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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/11, A61K 31/70, C07H 21/00 — A2

(11) International Publication Number: WO 96/27006

(43) International Publication Date: 6 September 1996 (06.09.96)

(21) International Application Number: PCT/US96/02840

(22) International Filing Date: 29 February 1996 (29.02.96)

(30) Priority Data:

08/398,945	2 March 1995 (02.03.95)	US
08/569,926	8 December 1995 (08.12.95)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: HUMAN VEGF-SPECIFIC ANTISENSE OLIGONUCLEOTIDES

(57) Abstract

Disclosed are oligonucleotides complementary to VEGF-specific nucleic acid useful in reducing the expression of VEGF. Also disclosed are pharmaceutical formulations containing such oligonucleotides and method useful for treating various disorders associated with neovascularization and angiogenesis.

HUMAN VEGF-SPECIFIC ANTISENSE OLIGONUCLEOTIDES

BACKGROUND OF THE INVENTION

This invention relates to neovascularization and vascular endothelial growth factor. More specifically, this invention relates to oligonucleotides specific for vascular endothelial growth factor nucleic acid and useful treatment of disorders that are associated with neovascularization and angiogenesis.

Neovascular diseases of the retina such as diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration are a major cause of blindness in the United States and the world, yet the biochemical events responsible for these processes have not been fully elucidated.

Diabetic retinopathy is the leading cause of blindness among working age adults (20-64) in the United States (Foster in *Harrison's Principles of Internal Medicine* (Isselbacher et al., eds.) McGraw-Hill, Inc., New York (1994) pp. 1994-1995). During the course of diabetes mellitus, the retinal vessels undergo changes that result in not only leaky vessels but also vessel drop out resulting in retinal hypoxia. The effects of these complications are hemorrhaging, "cotton wool" spots, retinal infarcts, and neovascularization of the retina resulting in bleeding and retinal detachment. If left untreated, there is a 60% chance of visual loss. Classic treatment for proliferative diabetic retinopathy is panretinal laser photocoagulation (PRP). However, complications can occur from panretinal laser photocoagulation such as foveal burns, hemorrhaging, retinal detachment, and choroidal vessel growth. Furthermore, other untoward effects of this treatment are decreased peripheral vision, decreased night vision, and changes in color perception (*Am. J. Ophthalmol.* (1976) **81**:383-396; *Ophthalmol.* (1991) **98**:741-840).

Thus, there is a need for a more effective treatment for diabetic retinopathy.

Retinopathy of prematurity (ROP) is a common cause of blindness in children in the United States (Pierce et al. (1994) *Int. Ophth. Clinics* 34:121-148). Premature babies are exposed to hyperoxic conditions after birth even without supplemental oxygen because the partial pressure of oxygen *in utero* is much lower than what is achieved when breathing normal room air. This relative hyperoxia is necessary for their survival yet can result in ROP. The blood vessels of the retina cease to develop into the peripheral retina resulting in ischemia and localized hypoxic conditions as the metabolic demands of the developing retina increase. The resulting hypoxia stimulates the subsequent neovascularization of the retina. This neovascularization usually regresses but can lead to irreversible vision loss. There are at least 10,000 new cases per year with a worldwide estimate of 10 million total cases. At present, there is no effective cure for ROP. Two therapeutic methods, cryotherapy and laser therapy, have been used but are not completely effective and themselves cause damage to the eye, resulting in a reduction of vision (Pierce et al. (1994) *Int. Ophth. Clinics* 34:121-148). Many other antiangiogenic compounds have been tested, but no inhibition in retinal neovascularization has been reported (Smith et al. (1994) *Invest. Ophthalmol. Vis. Sci.* 35:1442; Foley et al. (1994) *Invest. Ophthalmol. Vis. Sci.* 35:1442). Thus, there is a need for an effective treatment for ROP.

Age related macular degeneration is one of the leading causes of blindness in older adults in the United States, and may account for up to 30% of all bilateral blindness among Caucasian Americans (Anonymous (1994) *Prevent Blindness America*). This disease is characterized by loss of central vision, usually in both eyes, due to damage to retinal pigment epithelial cells which provide physiological support to the light sensitive photoreceptor cells of the retina. In most cases there is currently no effective treatment. In approximately 20% of exudative cases that are diagnosed early, laser treatment can prevent further loss of vision; however, this effect is temporary (Bressler et al.,

Principles and Practices of Ophthalmology (eds. Albert and Jakobiac), W.B. Saunders Co., Philadelphia, PA) (1994) Vol. 2 pp. 834-852).

Thus, there is a need for a more effective and permanent treatment for age related macular degeneration.

Ocular neovascularization is also the underlying pathology in sickle cell retinopathy, neovascular glaucoma, retinal vein occlusion, and other hypoxic diseases. These eye diseases as well as other pathological states associated with neovascularization (i.e., tumor growth, wound healing) appear to have hypoxia as a common factor (Knighton et al. (1983) *Science* 221:1283-1285; Folkman et al. (1987) *Science* 235:442-446; Klagsbrun et al. (1991) *Ann. Rev. Physiol.* 53:217-239; Miller et al. (1993) *Principles and Practice of Ophthalmology*, W.B. Saunders, Philadelphia, pp. 760; and Aiello et al. (1994) *New Eng. J. Med.* 331:1480-1487). Moreover, retinal neovascularization has been hypothesized to be the result of a "vasoformative factor" which is released by the retina in response to hypoxia (Michaelson (1948) *Trans. Ophthalmol. Soc. U. K.* 68:137-180; and Ashton et al. (1954) *Br. J. Ophthalmol.* 38:397-432). Recent experimental data show a high correlation between vascular endothelial growth factor expression and retinal neovascularization (Aiello et al. (1994) *New Eng. J. Med.* 331:1480-1487). Furthermore, elevated levels of vascular endothelial growth factor have recently been found in vitreous from patients with diabetes (Aiello et al., *ibid.*). Thus, this cytokine/growth factor may play an important role in neovascularization-related disease.

Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) is an endothelial cell-specific mitogen which has recently been shown to be stimulated by hypoxia and required for tumor angiogenesis (Senger et al. (1986) *Cancer* 46:5629-5632; Kim et al. (1993) *Nature* 362:841-844; Schweiki et al. (1992) *Nature* 359:843-845; Plate et al. (1992) *Nature* 359:845-848). It is a 34-

43 kD (with the predominant species at about 45 kD) dimeric, disulfide-linked glycoprotein synthesized and secreted by a variety of tumor and normal cells. In addition, cultured human retinal cells such as pigment epithelial cells and pericytes have been demonstrated to secrete VEGF and to increase VEGF gene expression in response to hypoxia (Adamis et al. (1993) *Biochem. Biophys. Res. Commun.* **193**:631-638; Plouet et al. (1992) *Invest. Ophthalmol. Vis. Sci.* **34**:900; Adamis et al. (1993) *Invest. Ophthalmol. Vis. Sci.* **34**:1440; Aiello et al. (1994) *Invest. Ophthalmol. Vis. Sci.* **35**:1868; Simorre-Pinatel et al. (1994) *Invest. Ophthalmol. Vis. Sci.* **35**:3393-3400). In contrast, VEGF in normal tissues is relatively low. Thus, VEGF appears to play a principle role in many pathological states and processes related to neovascularization. Regulation of VEGF expression in tissues affected by the various conditions described above could therefore be key in treatment or preventative therapies associated with hypoxia.

New chemotherapeutic agents termed "antisense oligonucleotides" have been developed which are capable of modulating cellular and foreign gene expression (see, Zamecnik et al. (1978) *Proc. Natl. Acad. Sci.* (USA) **75**:280-284). Without being limited to any theory or mechanism, it is generally believed that the activity of antisense oligonucleotides depends on the binding of the oligonucleotide to the target nucleic acid (e.g. to at least a portion of a genomic region, gene or mRNA transcript thereof), thus disrupting the function of the target, either by hybridization arrest or by destruction of target RNA by RNase H (the ability to activate RNase H when hybridized to RNA).

VEGF-specific antisense oligonucleotides have been developed (Uchida et al. (1995) *Antisense Res. & Dev.* **5(1)**:**87** (Abstract OP-10); Nomura et al., (1995) *Antisense Res. & Dev.* **5(1)**:**91** (Abstract OP-18)), although none have been demonstrated to reverse neovascularization or angiogenesis. Thus, a need still remains for the development of oligonucleotides that are capable of

reducing VEGF expression, and ultimately, of inhibiting the onset of diseases and disorders associated with the expression of VEGF.

SUMMARY OF THE INVENTION

It is known that cells affected by hypoxia induce VEGF. It has now been discovered that synthetic oligonucleotides specific for the mRNA for VEGF can inhibit hypoxia-associated neovascularization. It has also been discovered that oligonucleotides specific for nucleotides 58 to 90 of the VEGF gene can reduce the hypoxia-induced expression of VEGF mRNA and protein. This information has been exploited to develop the present invention which includes VEGF-specific oligonucleotides, pharmaceutical formulations, methods of reducing the expression of VEGF mRNA and protein, and methods of reducing neovascularization and of treating disorders and diseases related to neovascularization. As used herein, the term "neovascularization" refers to the growth of blood vessels and capillaries.

In one aspect, the invention provides synthetic oligonucleotides specific for human vascular endothelial growth factor nucleic acid and effective in inhibiting the expression of vascular endothelial growth factor is administered to a neovascularized tissue. This tissue may be a culture or may be part or the whole body of an animal such as a human or other mammal. In one embodiment, this invention provides synthetic oligonucleotides complementary to human VEGF-specific nucleic acids, and having a nucleic acid sequence set forth in the Sequence Listing as SEQ ID NOS:2-16.

As used herein, the term "synthetic oligonucleotide" refers to chemically synthesized polymers of nucleotides covalently attached via at least one 5' to 3' internucleotide linkage. In some embodiments, these oligonucleotides contain at least one deoxyribonucleotide, ribonucleotide, or both deoxyribonucleotides and ribonucleotides. In other embodiments, the synthetic

oligonucleotides used in the methods of the invention are from about 14 to about 28, or about 15 to about 30 nucleotides in length. In some preferred embodiments, these oligonucleotides contain from about 15 to about 25 nucleotides, and in other embodiments, from about 16 to 29 nucleotides.

For purposes of the invention, the term "oligonucleotide sequence that is complementary to a genomic region or an RNA molecule transcribed therefrom" is intended to mean an oligonucleotide that binds to the nucleic acid sequence under physiological conditions, e.g., by Watson-Crick base pairing (interaction between oligonucleotide and single-stranded nucleic acid) or by Hoogsteen base pairing (interaction between oligonucleotide and double-stranded nucleic acid) or by any other means including in the case of a oligonucleotide binding to RNA, causing pseudoknot formation. Binding by Watson-Crick or Hoogsteen base pairing under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence.

In some embodiments, the synthetic oligonucleotides of the invention are modified in a number of ways without compromising their ability to hybridize to nucleotide sequences contained within the mRNA for VEGF. The term "modified oligonucleotide" as used herein describes an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester linkage between the 5′ end of one nucleotide and the 3′ end of another nucleotide in which the 5′ nucleotide phosphate has been replaced with any number of chemical groups.

In some preferred embodiments, at least one internucleotide linkage of the oligonucleotide is an alkylphosphonate, phosphorothioate, phosphorodithioate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, and/or carboxymethyl ester.

The term "modified oligonucleotide" also encompasses oligonucleotides having at least one nucleotide with a modified base and/or sugar such as a 2'-O-substituted ribonucleotide. For purposes of the invention, the term "2'-O-substituted" means substitution of the 2' position of the pentose moiety with an -O-lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, e.g., with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups; or with a hydroxy, an amino or a halo group, but not with a 2'-H group. In some embodiments the oligonucleotides of the invention include four or five ribonucleotides 2'-O-alkylated at their 5' terminus (i.e., 5' 2-0-alkylated ribonucleotides), and/or four or five ribonucleotides 2'-O-alkylated at their 3' terminus (i.e., 3' 2-0-alkylated ribonucleotides). In preferred embodiments, the nucleotides of the synthetic oligonucleotides are linked by a or at least one phosphorothioate internucleotide linkage. The phosphorothioate linkages may be mixed R_p and S_p enantiomers, or they may be stereoregular or substantially stereoregular in either R_p or S_p form (see Iyer et al. (1995) *Tetrahedron Asymmetry* 6:1051-1054).

In another aspect of the invnetion, a method of treating retinopathy of prematurity (ROP) is provided. This method comprises the step of administering to a subject afflicted with ROP a therapeutic amount of an oligonucleotide specific for vascular endothelial growth factor nucleic acid and effective in inhibiting the expression of vascular endothelial growth factor in the retina. In another aspect of the invention, a method of treating diabetic retinopathy is provided. This method includes administering to a subject afflicted with diabetic retinopathy a therapeutic amount of an oligonucleotide specific for vascular endothelial growth factor nucleic acid and effective in inhibiting the expression of VEGF in the retina.

In yet another aspect of the invention, a method of treating age-related macular degeneration (ARMD) is provided, which includes comprising the step of administering to a subject afflicted with ARMD a therapeutic amount of an oligonucleotide specific for vascular endothelial growth factor nucleic acid effective in inhibiting the expression of VEGF in the retina.

Another aspect of the invention is assessment of the role of VEGF in neovascularization and angiogenesis associated with disease states.

In another aspect, the invention provides a method of inhibiting VEGF expression. In this method, nucleic acid specific for VEGF is contacted with an oligonucleotide of the invention. As used herein, the term "nucleic acid" encompasses a genomic region or an RNA molecule transcribed therefrom. In some embodiments, the nucleic acid is mRNA.

Without being limited to any theory or mechanism, it is generally believed that the activity of oligonucleotides used in accordance with this invention depends on the hybridization of the oligonucleotide to the target nucleic acid (e.g. to at least a portion of a genomic region, gene or mRNA transcript thereof), thus disrupting the function of the target. Such hybridization under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence. Thus, a preferred oligonucleotide used in accordance with the invention is capable of forming a stable duplex (or triplex in the Hoogsteen pairing mechanism) with the target nucleic acid; activate RNase H thereby causing effective destruction of the target RNA molecule, and in addition is capable of resisting nucleolytic degradation (e.g. endonuclease and exonuclease activity) *in vivo*. A number of the modifications to oligonucleotides described above and others which are known in the art specifically and successfully address each of these preferred characteristics. Also provided by the present invention is a pharmaceutical composition comprising at least one

synthetic oligonucleotide of claim 1 in a physiologically acceptable carrier.

In some preferred embodiments of the methods of the invention described above, the oligonucleotide is administered locally (e.g., intraocularly or interlesionally) and/or systemically. The term "local administration" refers to delivery to a defined area or region of the body, while the term "systemic administration is meant to encompass delivery to the whole organism by oral ingestion, or by intramuscular, intravenous, subcutaneous, or intraperitoneal injection.

Another aspect of the invention includes pharmaceutical compositions capable of inhibiting neovascularization and thus are useful in the methods of the invention. These compositions include a synthetic oligonucleotide of the present invention which specifically inhibits the expression of vascular endothelial growth factor and a physiologically and/or pharmaceutically acceptable carrier.

The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 is a diagrammatic representation of the murine model for retinal neovascularization;

FIG. 2 is a graphic representation of the ability of oligonucleotides of the invention to inhibit neovascularization during retinopathy of prematurity;

FIG. 3 is a diagrammatic representation of the ELISA used to test the ability of human VEGF-specific oligonucleotides to inhibit the expression of VEGF;

FIG. 4 is a graphic representation of the results of an ELISA demonstrating the reduction in the expression of VEGF in human cells in the presence of human VEGF-specific oligonucleotides of the invention;

FIG. 5 is a graphic representation of the results of a Northern blot demonstrating the reduction in the expression of VEGF by human cells in the presence of varying concentrations of human VEGF-specific oligonucleotides of the invention;

FIG. 6 is a schematic representation of regions of the VEGF cDNA sequence that are targeted by oligonucleotides of the invention;

FIG. 7 is a graphic representation of ELISA results demonstrating the ability of oligonucleotides H3-I and H3-J to inhibit VEGF expression induced by cobalt chloride;

FIG. 8 is a graphic representation of ELISA results demonstrating the ability of oligonucleotides H3-D, H3-E, and H3-F to inhibit VEGF expression induced by cobalt chloride;

FIG. 9 is a graphic representation of ELISA results demonstrating the ability of oligonucleotides H3-G and H3-H to inhibit VEGF expression induced by cobalt chloride;

FIG. 10 is a graphic representation of ELISA results demonstrating the ability of oligonucleotides H3 and H3-I to inhibit VEGF expression induced by cobalt chloride in M21 human melanoma cells *in vitro*; and

FIG. 11 is a graphic representation of ELISA results demonstrating the ability of modified H3 oligonucleotides to inhibit VEGF expression induced by cobalt chloride (H3-K: all 2'-O-methylated phosphorothioate ribonucleotides; H3-L: five 5' 2'-O-alkylated phosphorothioate ribonucleotides, the remainder, phosphorothioate deoxyribonucleotides; H3-M: five 3' 2'-O-alkylated phosphorothioate ribonucleotides, the remainder, phosphorothioate deoxyribonucleotides; and H3-N: five 3' 2'-O-alkylated phosphorothioate ribonucleotides, five 5' 2'-O-alkylated phosphorothioate ribonucleotides, and the remainder, phosphorothioate deoxyribonucleotides).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patents, allowed applications, and references cited herein are hereby incorporated by reference.

The present invention provides synthetic antisense oligonucleotides specific for VEGF nucleic acid which are useful in treating diseases and disorders associated with neovascularization and angiogenesis, including retinal neovascularization.

Antisense oligonucleotide technology provides a novel approach to the inhibition of gene expression (see generally, Agrawal (1992) *Trends in Biotech.* **10**:152-158; Wagner (1994) *Nature* **372**:333-335; and Stein et al. (1993) *Science* **261**:1004-1012). By binding to the complementary nucleic acid sequence (the sense strand), antisense oligonucleotide are able to inhibit splicing and translation of RNA. In this way, antisense oligonucleotides are able to inhibit protein expression. Antisense oligonucleotides have also been shown to bind to genomic DNA, forming a triplex, and inhibit transcription. Furthermore, a 17mer base sequence statistically occurs only once in the human genome, and thus extremely precise targeting of specific sequences is possible with such antisense oligonucleotides.

It has been determined that the VEGF coding region is comprised of eight axons (Tischer et al. (1994) *J. Biol. Chem.* **266**:11947-11954). Three VEGF transcripts, 121, 165, and 189 amino acids long, have been observed, suggesting that an alternative splicing mechanism is involved (Leung et al. (1989) *Science* **246**:1306-1309; Tischer et al. (1991) *J. Biol. Chem.* **266**:11947-11954). More recently, a fourth VEGF transcript was discovered which has a length encoding 206 amino acids (Houck et al. (1991) *Mol. Endocrinol.* **5**:1806-1814). Transcripts analogous to the 121 and

165 amino acid polypeptides have been identified in the bovine system (Leung et al. (1989) *Science* **246**:1306-1309), and the transcript corresponding to the 165 amino acid transcript have also been identified in the rodent system (Conn et al. (1990) *Proc. Natl. Acad. Sci. (USA)* **87**:1323-1327); Senger et al. (1990) *Cancer Res.* **50**:1774-1778; Claffey et al. (1992) *J. Biol. Chem.* **267**:16317-16322). Nucleic acid sequences encoding three forms of VEGF have also been reported in humans (Tischer et al. (1991) *J. Biol. Chem.* **266**:11947-11954), and comparisons between the human and the murine VEGF have revealed greater than 85% interspecies conservation (Claffey et al. (1992) *J. Biol. Chem.* **267**:16317-16322).

The oligonucleotides of the invention are directed to any portion of the VEGF nucleic acid sequence that effectively acts as a target for inhibiting VEGF expression. The sequence of the gene encoding VEGF has been reported in mice (Claffey et al., *ibid.*) and for humans (Tischer et al., *ibid.*). These targeted regions of the VEGF gene include any portions of the known exons. In addition, exon-intron boundaries are potentially useful targets for antisense inhibition of VEGF expression. The nucleotide sequences of some representative, non-limiting oligonucleotides specific for human VEGF are listed below in TABLE 1.

TABLE 1

OLIGO	TARGETED SITE	SEQUENCE (AS)	SEQ ID NO:
H-1	21-2	5'-CGCCGGGCCGCCAGCACACT-3'	1
H-1R	21-2	5'-CGCCGGGCCGCCAGCACACU-3'	2
H-1A	16-2	5'-GGCCGCCAGCACACT-3'	3
H-1B	26-2	5'-GCTCGCGCCGGGCCGCCAGCACACT-3'	4
H-2	76-57	5'-CAAGACAGCAGAAAGTTCAT-3'	5
H-3	80-62	5'-CACCCAAGACAGCAGAAAG-3'	6
H-3A	80-66	5'-CACCCAAGACAGCAG-3'	7
H-3B	86-62	5'-CCAATGCACCCAAGACAGCAGAAAG-3'	8
H-4	64-45	5'-AAGTTCATGGTTTCGGAGGC-3'	10
H-5	62-43	5'-GTTCATGGTTTCGGAGGCCC-3'	11
H-6	138-119	5'-GTGCAGCCTGGGACCACTTG-3'	12
H-7	628-609	5'-CGCCTCGGCTTGTCACATCT-3'	13
H-8	648-629	5'-CTTCCTCCTGCCCGGCTCAC-3'	14
H-8R	648-629	5'-CUUCCUCCUGCCCGGCUCAC-3'	15
H-8A	648-634	5'-CTTCCTCCTGCCCGG-3'	16
H-8B	653-629	5'-GGCTCCTTCCTCCTGCCCGGCTCAC-3'	17
H-9	798-779	5'-GTCTCCTCTTCCTTCATTTC-3'	18
H-9A	798-784	5'-GTCTCCTCTTCCTTC-3'	19
H-9B	803-779	5'-GCAGAGTCTCCTCTTCCTTCATTTC-3'	20
H-10	822-803	5'-CGGACCCAAAGTGCTCTGCG-3'	21
H-10A	817-803	5'-CCAAAGTGCTCTGCG-3'	22
H-10B	827-803	5'-CCCTCCGGACCCAAAGTGCTCTGCG-3'	23
H-11	E1-I1	5'-GGGCACGACCGCTTACCTTG-3'	24
H-12	I1-E2	5'-GGGACCACTGAGGACAGAAA-3'	25
H-13	I2-E3	5'-CACCACTGCATGAGAGGCGA-3'	26
H-14	E3-I3	5'-TCCCAAAGATGCCCACCTGC-3'	27
H-15	I3-E4	5'-CGCATAATCTGGAAAGGAAG-3'	28
H-17	59-40	5'-CATGGTTTCGGAGGCCCGAC-3'	30
H-17B	59-40	5'-CAUGGTTUCGGAGGCCCGAC-3'	31
H-18	61-42	5'-TTCATGGTTTCGGAGGCCCG-3'	32
E1/I1	E1/I1	5'-GACCGCTTACCTTGGCATGG-3'	33
I1/E2	I1/E2	5'-CCTGGGACCACTGAGGACAG-3'	34
E2/I2	E2/I2	5'-GGGACTCACCTTCGTGATGA-3'	35
I2/E3	I2/E3	5'-GAACTTCACCACTGCATGAG-3'	36
E3/I3	E3/I3	5'-TCCCAAAGATGCCCACCTGC-3'	37
I3/E4	I3/E4	5'-GCATAATCTGGAAAGGAAGG-3'	38
E4/I4	E4/I4	5'-ACATCCTCACCTGCATTCAC-3'	39
E4/I4B	E4/I4	5'-ACATCCUCACCTGCAUUCAC-3'	40
I4/E5	I4/E5	5'-TTTCTTTGGTCTGCAATGGG-3'	41
E5/I5	E5/I5	5'-GGCCACTTACTTTTCTTGTC-3'	42
I5/E7	I5/E7	5'-CACAGGGACTGGAAAATAAA-3'	43
E7/I7	E7/I7	5'-GGGAACCAACCTGCAAGTAC-3'	44
I7/E8	I7/E8	5'-GTCACATCTGAGGGAAATGG-3'	45
VH	641-621	5'-CTGCCCGGCTCACCGCCTCGG-3	46
H-19	56-38	5'-GGTTTCGGAGGCCCGACCG-3'	50

With the published nucleic acid sequences and the disclosure provided herein, those of skill in the art will be able to identify, with without undue experimentation, other antisense nucleic acid sequences that inhibit VEGF expression. For example, other sequences targeted specifically to human VEGF nucleic acid can be selected based on their ability to be cleaved by RNAse H. One useful targeted region is around bases 58 to 90. The nucleotide sequences of some representative, non-limiting oligonucleotides specific for human VEGF have SEQ ID NOS:54-68.

The oligonucleotides of the invention are composed of ribonucleotides, deoxyribonucleotides, or a combination of both, with the 5′ end of one nucleotide and the 3′ end of another nucleotide being covalently linked. These oligonucleotides are at least 14 nucleotides in length, but are preferably 15 to 30 nucleotides long, with 15 to 29mers being the most common.

These oligonucleotides can be prepared by the art recognized methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer as described in Uhlmann et al. (*Chem. Rev.* (1990) 90:534-583) and Agrawal (*Trends Biotechnol. (1992)* 10:152-158).

The oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to VEGF mRNA. For example, the oligonucleotides may contain at least one or a combination of other than phosphodiester internucleotide linkages between the 5′ end of one nucleotide and the 3′ end of another nucleotide in which the 5′ nucleotide phosphodiester linkage has been replaced with any number of chemical groups. Examples of such chemical groups include alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters.

For example, U.S. Patent No. 5,149,797 describes traditional chimeric oligonucleotides having a phosphorothioate core region interposed between methylphosphonate or phosphoramidate flanking regions. U.S. Patent Application Ser. No. (47508-559), filed on August 9, 1995, discloses "inverted" chimeric oligonucleotides comprising one or more nonionic oligonucleotide region (e.g. alkylphosphonate and/or phosphoramidate and/or phosphotriester internucleoside linkage) flanked by one or more region of oligonucleotide phosphorothioate. Various oligonucleotides with modified internucleotide linkages can be prepared according to known methods (see, e.g., Goodchild (1990) *Bioconjugate Chem.* **2**:165-187; Agrawal et al., (1988) *Proc. Natl. Acad. Sci. (USA)* **85**:7079-7083; Uhlmann et al. (1990) *Chem. Rev.* **90**:534-583; and Agrawal et al. (1992) *Trends Biotechnol.* **10**:152-158.

The phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be stereoregular or substantially stereoregular in either Rp or Sp form (see Iyer et al. (1995) *Tetrahedron Asymmetry* **6**:1051-1054). Oligonucleotides with phosphorothioate linkages can be prepared using methods well known in the field such as phosphoramidite (see, e.g., Agrawal et al. (1988) *Proc. Natl. Acad. Sci. (USA)* **85**:7079-7083). or by H-phosphonate (see, e.g., Froehler (1986) *Tetrahedron Lett.* **27**:5575-5578) chemistry. The synthetic methods described in Bergot et al. (*J. Chromatog.* (1992) **559**:35-42) can also be used.

Oligonucleotides which are self-stabilized are also considered to be modified oligonucleotides useful in the methods of the invention (Tang et al. (1993) *Nucleic Acids Res.* **20**:2729-2735). These oligonucleotides comprise two regions: a target hybridizing region; and a self-complementary region having an oligonucleotide sequence complementary to a nucleic acid sequence that is within the self-stabilized oligonucleotide.

Other modifications include those which are internal or at the end(s) of the oligonucleotide molecule and include additions

to the molecule of the internucleoside phosphate linkages, such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position).

Other examples of modifications to sugars include modifications to the 2' position of the ribose moiety which include but are not limited to 2'-O-substituted with an -O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl, or allyl group having 2-6 carbon atoms wherein such -O-alkyl, aryl or allyl group may be unsubstituted or may be substituted, (e.g., with halo, hydroxy, trifluoromethyl cyano, nitro acyl acyloxy, alkoxy, carboxy, carbalkoxyl, or amino groups), or with an amino, or halo group. None of these substitutions are intended to exclude the native 2'-hydroxyl group in the case of ribose or 2'-H- in the case of deoxyribose. PCT Publication No. WO 94/02498 discloses traditional hybrid oligonucleotides having regions of 2'-O-substituted ribonucleotides flanking a DNA core region. U.S. Patent Application Serial No. (47508-559), filed August 9, 1995, discloses an "inverted" hybrid oligonucleotide which includes an oligonucleotide comprising a 2'-O-substituted (or 2' OH, unsubstituted) RNA region which is in between two oligodeoxyribonucleotide regions, a structure that "inverted relative to the "traditional" hybrid oligonucleotides. Nonlimiting examples of particularly useful oligonucleotides of the invention have 2'-O-alkylated ribonucleotides at their 3', 5', or 3' and 5' termini, with at least four or five contiguous nucleotides being so modified. Non-limiting examples of 2'-O-

alkylated groups include 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, and 2'-O-butyls.

Other modified oligonucleotides are capped with a nuclease resistance-conferring bulky substituent at their 3' and/or 5' end(s) , or have a substitution in one nonbridging oxygen per nucleotide. Such modifications can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule.

A nonlimiting list of useful unmodified and modified oligonucleotides of the invention are listed below in Table 2.

TABLE 2

OLIGO	TARGETED SITE	(5' → 3')	SEQ ID NO:
H-3Ia1	81-62	GCACCCAAGACAGCAGAAAG	55
H-3Ia2	81-62	**GCACC**CAAGACAGCAGAAAG	55
H-3Ia3	81-62	GCACCCAAGACAGCA**GAAAG**	55
H-3Ia4	81-62	**GCACC**CAAGACAGCA**GAAAG**	55
H-3Ia5	81-62	**GCAC**CCAAGACAGCAGAAAG	55
H-3Ia6	81-62	GCACCCAAGACAGCAG**AAAG**	55
H-3Ia7	81-62	**GCAC**CCAAGACAGCAG**AAAG**	55
H-3Ia8	81-62	**GCACC**CAAGACAGCAG**AAAG**	55
H-3Ia9	81-62	**GCAC**CCAAGACAGCA**GAAAG**	55
H-3Ia10	81-62	**GCACCCAAGACAGCAGAAAG**	55
H-3I1	82-62	TGCACCCAAGACAGCAGAAAG	56
H-3I2	82-62	**TGCAC**CCAAGACAGCAGAAAG	56
H-3I3	82-62	TGCACCCAAGACAGCA**GAAAG**	56
H-3I4	82-62	**TGCAC**CCAAGACAGCA**GAAAG**	56
H-3I5	82-62	**TGCA**CCCAAGACAGCAGAAAG	56
H-3I6	82-62	TGCACCCAAGACAGCAG**AAAG**	56
H-3I7	82-62	**TGCA**CCCAAGACAGCAG**AAAG**	56
H-3I8	82-62	**TGCAC**CCAAGACAGCAG**AAAG**	56
H-3I9	82-62	**TGCA**CCCAAGACAGCA**GAAAG**	56
H-3I10	82-62	**TGCACCCAAGACAGCAGAAAG**	56
H-3Ja1	83-62	ATGCACCCAAGACAGCAGAAAG	57
H-3Ja2	83-62	**ATGCA**CCCAAGACAGCAGAAAG	57
H-3Ja3	83-62	ATGCACCCAAGACAGCA**GAAAG**	57
H-3Ja4	83-62	**ATGCA**CCCAAGACAGCA**GAAAG**	57
H-3Ja5	83-62	**ATG**CACCCAAGACAGCAGAAAG	57
H-3Ja6	83-62	ATGCACCCAAGACAGCAG**AAAG**	57
H-3Ja7	83-62	**ATG**CACCCAAGACAGCAG**AAAG**	57
H-3Ja8	83-62	**ATGCA**CCCAAGACAGCA**GAAAG**	57
H-3Ja9	83-62	**ATGCA**CCCAAGACAGCAG**AAAG**	57
H-3Ja10	83-62	**ATGCACCCAAGACAGCAGAAAG**	57
H-3J1	84-62	AATGCACCCAAGACAGCAGAAAG	58
H-3J2	84-62	**AATG**CACCCAAGACAGCAGAAAG	58

TABLE 2 (conti'.)

OLIGO	TARGETED SITE	SEQUENCE (5' → 3')	SEQ ID NO:
H-3J3	84-62	AATGCACCCAAGACAGCA**GAAAG**	58
H-3J4	84-62	**AATG**CACCCAAGACAGCA**GAAAG**	58
H-3J5	84-62	**AATG**CACCCAAGACAGCAGAAAG	58
H-3J6	84-62	AATGCACCCAAGACAGCAG**AAAG**	58
H-3J7	84-62	**AATG**CACCCAAGACAGCAG**AAAG**	58
H-3J8	84-62	**AATGC**ACCCAAGACAGCAG**AAAG**	58
H-3J9	84-62	**AATG**CACCCAAGACAGCA**GAAAG**	58
H-3J10	84-62	**AATGCACCCAAGACAGCAGAAAG**	58
H-3Xa1	85-62	CAATGCACCCAAGACAGCAGAAAG	59
H-3Xa2	85-62	**CAATG**CACCCAAGACAGCAGAAAG	59
H-3Xa3	85-62	CAATGCACCCAAGACAGCA**GAAAG**	59
H-3Xa4	85-62	**CAATG**CACCCAAGACAGCA**GAAAG**	59
H-3Xa5	85-62	**CAAT**GCACCCAAGACAGCAGAAAG	59
H-3Xa6	85-62	CAATGCACCCAAGACAGCAG**AAAG**	59
H-3Xa7	85-62	**CAAT**GCACCCAAGACAGCAG**AAAG**	59
H-3Xa8	85-62	**CAATG**CACCCAAGACAGCAG**AAAG**	59
H-3Xa9	85-62	**CAAT**GCACCCAAGACAGCA**GAAAG**	59
H-3Xa10	85-62	**CAATGCACCCAAGACAGCAGAAAG**	59
H-3X1	86-62	CCAATGCACCCAAGACAGCAGAAAG	60
H-3X2	86-62	**CCAAT**GCACCCAAGACAGCAGAAAG	60
H-3X3	86-62	CCAATGCACCCAAGACAGCA**GAAAG**	60
H-3X4	86-62	**CCAAT**GCACCCAAGACAGCA**GAAAG**	60
H-3X5	86-62	**CCAA**TGCACCCAAGACAGCAGAAAG	60
H-3X6	86-62	CCAATGCACCCAAGACAGCAG**AAAG**	60
H-3X7	86-62	**CCAA**TGCACCCAAGACAGCAG**AAAG**	60
H-3X8	86-62	**CCAAT**GCACCCAAGACAGCAG**AAAG**	60
H-3X9	86-62	**CCAA**TGCACCCAAGACAGCA**GAAAG**	60
H-3X10	86-62	**CCAATGCACCCAAGACAGCAGAAAG**	60
H-3Ya1	87-62	TCCAATGCACCCAAGACAGCAGAAAG	61
H-3Ya2	87-62	**TCCAA**TGCACCCAAGACAGCAGAAAG	61
H-3Ya3	87-62	TCCAATGCACCCAAGACAGCA**GAAAG**	61
H-3Ya4	87-62	**TCCAA**TGCACCCAAGACAGCA**GAAAG**	61
H-3Ya5	87-62	**TCCA**ATGCACCCAAGACAGCAGAAAG	61
H-3Ya6	87-62	TCCAATGCACCCAAGACAGCAG**AAAG**	61
H-3Ya7	87-62	**TCCA**ATGCACCCAAGACAGCAG**AAAG**	61
H-3Ya8	87-62	**TCCAA**TGCACCCAAGACAGCAG**AAAG**	61
H-3Ya9	87-62	**TCCA**ATGCACCCAAGACAGCA**GAAAG**	61
H-3Ya10	87-62	**TCCAATGCACCCAAGACAGCAGAAAG**	61
H-Y1	88-62	CTCCAATGCACCCAAGACAGCAGAAAG	62
H-Y2	88-62	**CTCCA**ATGCACCCAAGACAGCAGAAAG	62
H-Y3	88-62	CTCCAATGCACCCAAGACAGCA**GAAAG**	62
H-Y4	88-62	**CTCCA**ATGCACCCAAGACAGCA**GAAAG**	62
H-Y5	88-62	**CTCC**AATGCACCCAAGACAGCAGAAAG	62
H-Y6	88-62	CTCCAATGCACCCAAGACAGCAG**AAAG**	62
H-Y7	88-62	**CTCC**AATGCACCCAAGACAGCAG**AAAG**	62
H-Y8	88-62	**CTCCA**ATGCACCCAAGACAGCAG**AAAG**	62
H-Y9	88-62	**CTCC**AATGCACCCAAGACAGCA**GAAAG**	62
H-Y10	88-62	**CTCCAATGCACCCAAGACAGCAGAAAG**	62
H-Za1	89-62	GCTCCAATGCACCCAAGACAGCAGAAAG	63
H-Za2	89-62	**GCTCC**AATGCACCCAAGACAGCAGAAAG	63
H-Za3	89-62	GCTCCAATGCACCCAAGACAGCA**GAAAG**	63

TABLE 2 (conti'.)

OLIGO	TARGETED SITE	SEQUENCE (5' → 3')	SEQ ID NO:
H-Za4	89-62	**GCTC**CAATGCACCCAAGACAGCA**GAAAG**	63
H-Za5	89-62	**GCTC**CAATGCACCCAAGACAGCAGAAAG	63
H-Za6	89-62	GCTCCAATGCACCCAAGACAGCAG**AAAG**	63
H-Za7	89-62	**GCTC**CAATGCACCCAAGACAGCAG**AAAG**	63
H-Za8	89-62	**GCTC**CAATGCACCCAAGACAGCAG**AAAG**	63
H-Za9	89-62	**GCTC**CAATGCACCCAAGACAGCA**GAAAG**	63
H-Za10	89-62	**GCTCCAATGCACCCAAGACAGCAGAAAG**	63
H-Z1	90-62	GGCTCCAATGCACCCAAGACAGCAGAAAG	64
H-Z2	90-62	**GGCTC**CAATGCACCCAAGACAGCAGAAAG	64
H-Z3	90-62	GGCTCCAATGCACCCAAGACAGCA**GAAAG**	64
H-Z4	90-62	**GGCTC**CAATGCACCCAAGACAGCA**GAAAG**	64
H-Z5	90-62	**GGCT**CCAATGCACCCAAGACAGCAGAAAG	64
H-Z6	90-62	GGCTCCAATGCACCCAAGACAGCAG**AAAG**	64
H-Z7	90-62	**GGCT**CCAATGCACCCAAGACAGCAG**AAAG**	64
H-Z8	90-62	**GGCTC**CAATGCACCCAAGACAGCAG**AAAG**	64
H-Z9	90-62	**GGCT**CCAATGCACCCAAGACAGCA**GAAAG**	64
H-Z10	90-62	**GGCTCCAATGCACCCAAGACAGCAGAAAG**	64
H-3D1	80-63	CACCCAAGACAGCAGAAA	65
H-3D2	80-63	**CACC**CAAGACAGCAGAAA	65
H-3D3	80-63	CACCCAAGACAGC**AGAAA**	65
H-3D4	80-63	**CACC**CAAGACAGC**AGAAA**	65
H-3D5	80-63	**CACC**CAAGACAGCAGAAA	65
H-3D6	80-63	CACCCAAGACAGCA**GAAA**	65
H-3D7	80-63	**CACC**CAAGACAGCA**GAAA**	65
H-3D8	80-63	**CACC**CAAGACAGCA**GAAA**	65
H-3D9	80-63	**CACC**CAAGACAGC**AGAAA**	65
H-3D10	80-63	**CACCCAAGACAGCAGAAA**	65
H-3E1	80-64	CACCCAAGACAGCAGAA	66
H-3E2	80-64	**CACC**CAAGACAGCAGAA	66
H-3E3	80-64	CACCCAAGACAG**CAGAA**	66
H-3E4	80-64	**CACC**CAAGACAG**CAGAA**	66
H-3E5	80-64	**CACC**CAAGACAGCAGAA	66
H-3E6	80-64	CACCCAAGACAG**CAGAA**	66
H-3E7	80-64	**CACC**CAAGACAG**CAGAA**	66
H-3E8	80-64	**CACC**CAAGACAG**CAGAA**	66
H-3E9	80-64	**CACC**CAAGACAG**CAGAA**	66
H-3E10	80-64	**CACCCAAGACAGCAGAA**	66
H-3F1	80-65	CACCCAAGACAGCAGA	67
H-3F2	80-65	**CACC**CAAGACAGCAGA	67
H-3F3	80-65	CACCCAAGACA**GCAGA**	67
H-3F4	80-65	**CACC**CAAGACA**GCAGA**	67
H-3F5	80-65	**CACC**CAAGACAGCAGA	67
H-3F6	80-65	CACCCAAGACAG**CAGA**	67
H-3F7	80-65	**CACC**CAAGACAG**CAGA**	67
H-3F8	80-65	**CACC**CAAGACAG**CAGA**	67
H-3F9	80-65	**CACC**CAAGACA**GCAGA**	67
H-3F10	80-65	**CACCCAAGACAGCAGA**	67
H-3G1	80-60	CACCCAAGACAGCAGAAAGTT	68
H-3G2	80-60	**CACC**CAAGACAGCAGAAAGTT	68
H-3G3	80-60	CACCCAAGACAGCAGA**AAGTT**	68
H-3G4	80-60	**CACC**CAAGACAGCAGA**AAGTT**	68

TABLE 2 (conti'.)

OLIGO	TARGETED SITE	SEQUENCE (5' → 3')	SEQ ID NO:
H-3G5	80-60	**CACC**CAAGACAGCAGAAAGTT	68
H-3G6	80-60	CACCCAAGACAGCAGAA**AGTT**	68
H-3G7	80-60	**CACC**CAAGACAGCAGAA**AGTT**	68
H-3G8	80-60	**CACCC**AAGACAGCAGA**AAGTT**	68
H-3G9	80-60	**CACC**CAAGACAGCAGA**AAGTT**	68
H-3G10	80-60	**CACCCAAGACAGCAGAAAGTT**	68
H-3H1	80-58	CACCCAAGACAGCAGAAAGTTCAT	69
H-3H2	80-58	**CACC**CAAGACAGCAGAAAGTTCAT	69
H-3H3	80-58	CACCCAAGACAGCAGAAAGT**TCAT**	69
H-3H4	80-58	**CACC**CAAGACAGCAGAAAGT**TCAT**	69
H-3H5	80-58	**CACCC**AAGACAGCAGAAAGTTCAT	69
H-3H6	80-58	CACCCAAGACAGCAGAAAGTT**CAT**	69
H-3H7	80-58	**CACC**CAAGACAGCAGAAAGT**TCAT**	69
H-3H8	80-58	**CACC**CAAGACAGCAGAAAGT**TCAT**	69
H-3H9	80-58	**CACC**CAAGACAGCAGAAAGT**TCAT**	69
H-3H10	80-58	**CACCCAAGACAGCAGAAAGTTCAT**	69

Preferably, the nucleotides bolded in the oligonucleotides in Table 2 above are 2'-0-alkylated, and all of the nucleotides are linked via non-phosphodiester internucleotide linkages such as phosphorothioates.

The preparation of these modified oligonucleotides is well known in the art (reviewed in Agrawal et al.(1992) *Trends Biotechnol.* **10**:152-158); Agrawal et al.(1995) *Curr. Opin. Biotechnol.* **6**:12-19). For example, nucleotides can be covalently linked using art-recognized techniques such as phosphoramidate, H-phosphonate chemistry, or methylphosphoramidate chemistry (*see, e.g.*, Uhlmann et al. (1990) *Chem. Rev.* **90**:543-584; Agrawal et al. (1987) *Tetrahedron. Lett.* **28**:(31):3539-3542); Caruthers et al. (1987) *Meth. Enzymol.* **154**:287-313; U.S. Patent 5,149,798). Oligomeric phosphorothioate analogs can be prepared using methods well known in the field such as methoxyphosphoramidite (*see, e.g.*, Agrawal et al. (1988) *Proc. Natl. Acad. Sci. (USA)* **85**:7079-7083) or H-phosphonate (*see, e.g.*, Froehler (1986) *Tetrahedron Lett.* **27**:5575-5578) chemistry. The synthetic methods described in Bergot et al. (*J. Chromatog.* (1992) **559**:35-42) can also be used.

The synthetic antisense oligonucleotides of the invention in the form of a therapeutic formulation are useful in treating diseases, and disorders, and conditions associated with angiogenesis and neovascularization including, but not limited to, retinal neovascularization, tumor growth, and wound healing. In such methods, a therapeutic amount of a synthetic oligonucleotide of the invention and effective in inhibiting the expression of vascular endothelial growth factor is administered to a cell. This cell may be part of a cell culture, a neovascularized tissue culture, or may be part or the whole body of an animal such as a human or other mammal. Administration may be bolus, intermittent, or continuous, depending on the condition and response, as determined by those with skill in the art. In some preferred embodiments of the methods of the invention described above, the oligonucleotide is administered locally (e.g., intraocularly or interlesionally) and/or systemically. The term "local administration" refers to delivery to a defined area or region of the body, while the term "systemic administration is meant to encompass delivery to the whole organism by oral ingestion, or by intramuscular, intravenous, subcutaneous, or intraperitoneal injection.

Such methods can be used to treat retinopathy of prematurity (ROP), diabetic retinopathy, age-related macular degeneration, sickle cell retinopathy, neovascular glaucoma, retinal vein occlusion, and other hypoxic diseases.

The synthetic oligonucleotides of the invention may be used as part of a pharmaceutical composition when combined with a physiologically and/or pharmaceutically acceptable carrier. The characteristics of the carrier will depend on the route of administration. Such a composition may contain, in addition to the synthetic oligonucleotide and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance inhibition of VEGF expression or which will reduce

neovascularization. For example, combinations of synthetic oligonucleotides, each of which is directed to different regions of the VEGF mRNA, may be used in the pharmaceutical compositions of the invention. The pharmaceutical composition of the invention may further contain nucleotide analogs such as azidothymidine, dideoxycytidine, dideosyinosine, and the like. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with the synthetic oligonucleotide of the invention, or to minimize side-effects caused by the synthetic oligonucleotide of the invention. Conversely, the synthetic oligonucleotide of the invention may be included in formulations of a particular anti-VEGF or anti-neovascularization factor and/or agent to minimize side effects of the anti-VEGF factor and/or agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which the synthetic oligonucleotides of the invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. One particularly useful lipid carrier is lipofectin. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323. The pharmaceutical composition of the invention may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells, as described by Zhao et al. (in press), or slow release polymers.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show

a meaningful patient benefit, i.e., healing of chronic conditions characterized by neovascularization or a reduction in neovascularization, itself, or in an increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of one, two, or more of the synthetic oligonucleotide oligonucleotides of the invention is administered to a subject afflicted with a disease or disorder related to neovascularization, or to a tissue which has been neovascularized. The synthetic oligonucleotide of the invention may be administered in accordance with the method of the invention either alone of in combination with other known therapies for neovascularization. When co-administered with one or more other therapies, the synthetic oligonucleotide of the invention may be administered either simultaneously with the other treatment(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the synthetic oligonucleotide of the invention in combination with the other therapy.

Administration of the synthetic oligonucleotide of the invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as intraocular, oral ingestion, inhalation, or cutaneous, subcutaneous, intramuscular, or intravenous injection.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered orally, the synthetic oligonucleotide will be in the form of a tablet,

capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide and preferably from about 1 to 50% synthetic oligonucleotide.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered by intravenous, subcutaneous, intramuscular, intraocular, or intraperitoneal injection, the synthetic oligonucleotide will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, subcutaneous, intramuscular, intraperitoneal, or intraocular injection should contain, in addition to the synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend

upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patent has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 10 μg to about 20 mg of synthetic oligonucleotide per kg body or organ weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Some diseases lend themselves to acute treatment while others require to longer term therapy. Proliferative retinopathy can reach a threshold in a matter of days as seen in ROP, some cases of diabetic retinopathy, and neovascular glaucoma. Premature infants are at risk for neovascularization around what would be 35 weeks gestation, a few weeks after birth, and will remain at risk for a short period of time until the retina becomes vascularized. Diabetic retinopathy can be acute but may also smolder in the proliferative phase for considerably longer. Diabetic retinopathy will eventually become quiescent as the vasoproliferative signal diminishes with neovascularization or destruction of the retina.

Both acute and long term intervention in retinal disease are worthy goals. Intravitreal injections of oligonucleotides

against VEGF can be an effective means of inhibiting retinal neovascularization in an acute situation. However for long term therapy over a period of years, systemic delivery (intraperitoneal, intramuscular, subcutaneous, intravenous) either with carriers such as saline, slow release polymers, or liposomes should be considered.

In some cases of chronic neovascular disease, systemic administration of oligonucleotides may be preferable. Since the disease process concerns vessels which are abnormal and leaky, the problem of passage through the blood brain barrier may not be a problem. Therefore, systemic delivery may prove efficacious. The frequency of injections is from continuous infusion to once a month, depending on the disease process and the biological half life of the oligonucleotides.

In addition to inhibiting neovascularization *in vivo*, antisense oligonucleotides specific for VEGF are useful in determining the role of this cytokine in processes where neovascularization is involved. For example, this technology is useful in *in vitro* systems which mimic blood vessel formation and permeability, and in *in vivo* system models of neovascularization, such as the murine model described below.

A murine model of oxygen-induced retinal neovascularization has been established which occurs in 100% of treated animals and is quantifiable (Smith et al. (1994) *Invest. Ophthalmol. Vis. Sci.* **35**:101-111). Using this model, a correlation has been determined between increasing expression of VEGF message and the onset of retinal neovascularization in the inner nuclear and ganglion cell layers (i.e., in Müller cells) (Pierce et al. (1995) *Proc. Natl. Acad. Sci. (USA)* (in press). This result has been confirmed by Northern blot and *in situ* hybridization analysis of whole retinas at different time points during the development of neovascularization (Pierce et al., *ibid.*).

Oligonucleotides of the invention are also useful in a method of reducing the expression of VEGF. The target VEGF expression can be in vitro or in any cell which expresses VEGF. In this method, nucleic acid specific for VEGF is contacted with an oligonucleotide of the invention such that transcription of the nucleic acid to mRNA and/or protein is reduced or inhibited.

That oligonucleotides of the invention can inhibit VEGF expression at the protein level can be demonstrated using an ELISA which specifically detects human VEGF and a VEGF-expressing cell line such as a human glioblastoma (e.g., U373 ATCC Ac. no. HTB17, American Type Culture Collection, Rockville, MD) or a human melanoma (e.g., SK-MEL-2, ATCC Ac. no. HTB68, American Type Culture Collection, Rockville, MD; or M21). Briefly, when a human glioblastoma cell line U373 and a human melanoma cell line M21 were treated with VEGF-specific oligonucleotides of the invention, these cells stop expressing VEGF in a sequence-specific manner, as shown in FIGS. 7, 8, 9, and 10, respectively. FIG. 11 demonstrates that modification of the oligonucleotides does not reduce their inhibitory activity. Oligonucleotides of the invention also reduced VEGF mRNA expression, as demonstrated by the Northern analyses described in EXAMPLE 4 below.

The role of VEGF in tumor formation *in vivo* can be demonstrated using an athymic mouse injected with as an animal model. M21 cells are known to generate palpable tumors in mice in about 1 to 1.5 weeks. Alternately, a U373 cell line which has been passed through an athymic mouse in the presence of Engelbreth Holm Swarm (EMS) tumor matrix (Matrigel™, Collaborative Research, Waltham, MA) may be used. When mice are injected with VEGF-specific oligonucleotides of the invention, there will be a reduction in tumor weight and volume if VEGF expression is reduced by oligonucleotides or pharmaceutical formulations of the invention.

That VEGF plays a role in retinal neovascularization has been shown using the murine model of neovascularization described

above. Three independent experiments were performed using antisense oligonucleotides specific for VEGF (JG-3 (SEQ ID NO: 47), JG-4, (SEQ ID NO: 48), and Vm (SEQ ID NO: 46), and a corresponding sense oligonucleotide (V2 (SEQ ID NO: 49). These oligonucleotides were designed using the known nucleic sequence of murine VEGF (Claffee et al. (1992) *J. Biol. Chem.* **267**:16317-16322). The sequence of the Vm oligonucleotide (SEQ ID NO:46) is targeted to the sequence surrounding the translational TGA stop site (TGA). The sequence of JG-4 (SEQ ID NO:48) is targeted to the sequence 5′ to and containing the ATG of the translational start site of the murine VEGF molecule. The sequence of JG-3 (SEQ ID NO:47) is targeted to the 5′ untranslated region, and the V2 sense sequence is targeted to the sequence surrounding the translational start site (ATG). A compilation of the results of these experiments is presented in FIG. 2. These results indicate that Vm (SEQ ID NO:46) antisense oligonucleotide significantly reduces retinal neovascularization when compared with both untreated and sense oligonucleotide V2, (SEQ ID NO:49) controls. JG-3 (SEQ ID NO:47) and JG-4 (SEQ ID NO:47) show significant activity when compared against untreated eyes. The sense control oligonucleotide V2 (SEQ ID NO:49) does not show any significant activity when compared with untreated eyes.

In the studies described above, the human VEGF antisense oligonucleotide which corresponds to murine JG-3 is H-1 (SEQ ID NO:1), which is targeted to the 5′ untranslated region; that which corresponds to murine JG-4 is H-17 (SEQ ID NO:30), which is targeted to the sequence 5′ to and containing the ATG of the translational start site of the human VEGF molecule; and that which corresponds to the murine Vm gene is VH (SEQ ID NO:46), which is targeted to sequences surrounding the translational stop site (TGA) of the human VEGF molecule. These antisense oligonucleotides of the invention are expected to inhibit VEGF expression in human cells in much the same way as the murine antisense oligonucleotides inhibit expression of VEGF in mouse cells.

Human VEGF antisense sequences corresponding to other murine sequences are also known. For example, human oligonucleotide H-6 (SEQ ID NO:12) corresponds to a region spanning murine sequences JG-6 (SEQ ID NO:52) and JG-7 (SEQ ID NO:53), and human oligonucleotide H-2 (SEQ ID NO:5) is in the same region as murine sequence JG-5 (SEQ ID NO:51). It is likely that these sequences have a similar effect on inhibition of VEGF expression and hence on controlling neovascularization.

There are several methods by which the effects of antisense oligonucleotides on VEGF expression and neovascularization can be monitored. One way is a capture ELISA developed for quantifying human VEGF protein expressed by cells. Using this assay, it has been determined that an antisense phosphorothioate oligonucleotide H-3 (SEQ ID NO:6) targeted to a sequence just 3' to the translational start site can inhibit the hypoxic induction of VEGF expression in a sequence-specific manner, compared with random (R) and sense (H-16, SEQ ID NO:29) controls), as shown in FIG. 4. This inhibition is reproducible and in this in vitro system appears to be lipid carrier-specific and antisense-specific as only antisense oligonucleotide H-3 (SEQ ID NO:6) in the presence of lipofectin (a lipid carrier), and not lipofectamine (another lipid carrier), results in inhibition of VEGF protein expression.

At the RNA level, Northern blots (Sambrook et al. (1989) *Molecular Cloning; a Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY, Vol. 1, pp. 7.38; Arcellana-Panlilio et al. (1993) *Meth. Enz.* 225:303-328) can be performed to determine the extent that oligonucleotides of the invention inhibit the expression of VEGF mRNA. For example, as shown in FIG. 5, a histogram representing Northern blot analysis demonstrates a decrease in VEGF RNA levels in culture human cells treated with antisense oligonucleotide H-3 (SEQ ID NO:6), while there is only a minimal change in VEGF RNA levels in samples treated with sense control H-16 (SEQ ID NO:29).

In addition, bioactivity can be determined by several methods, including the Miles vessel permeability assay (Miles and Miles (1952) *J. Physiol.* (Lond.) 118:228), which measures vessel permeability, endothelial cell mitogenicity, which measures cell growth, and intracellular calcium release in endothelial cells (see, e.g., Brock and Capasso (1988) *J. Cell. Physiol.* **136**:54), which measures the release of calcium in response to VEGF binding to its receptor on endothelial cells.

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLE 1

PREPARATION OF VEGF-SPECIFIC OLIGONUCLEOTIDES

Human VEGF cDNA is transcribed *in vitro* using an *in vitro* eukaryotic transcription kit (Stratagene, La Jolla, CA). The RNA is labelled with ^{32}P using T-4 polynucleotide kinase as described by (Sambrook et al. (1989) *Molecular Cloning; a Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY, Vol. 1, pp. 5.71). The labelled RNA is incubated in the presence of a randomer 20mer library and RNAse H, an enzyme which cleaves RNA-DNA duplexes (Boehringer Mannheim, Indianapolis, IN). Cleavage patterns are analyzed on a 6% polyacrylamide urea gel. The specific location of the cleaved fragments is determined using a human VEGF sequence ladder (Sequenase Kit, United States Biochemical, Cleveland, OH).

EXAMPLE 2

ANIMAL MODEL OF RETINAL NEOVASCULARIZATION

A. Preparation of Oligonucleotides

Synthesis of the following oligonucleotides: JG-3 (SEQ ID NO:47), JG-4 (SEQ ID NO:48), Vm (SEQ ID NO:46), and V2 (SEQ ID NO:49), was performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (see, e.g., Uhlmann et al. (*Chem. Rev.* (1990) **90**:534-583). Following assembly and deprotection, oligonucleotides were ethanol precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration.

The purity of these oligonucleotides was tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation was determined using the Luminous Amebocyte Assay (Bang (1953) *Biol. Bull.* (Woods Hole, MA) **105**:361-362).

B. Preparation of Animal Model

Seven day postnatal mice (P7, C57bl/6J, (Children's Hospital Breeding Facilities, Boston, MA) were exposed to 5 days of hyperoxic conditions (75 +/- 2%) oxygen in a sealed incubator connected to a Bird 3-M oxygen blender (flow rate: 1.5 liters/minute; Bird, Palm Springs, CA). The oxygen concentration was monitored by means of an oxygen analyzer (Beckman, Model D2, Irvine, CA). After 5 days (P12), the mice were returned to room air. Maximal retinal neovascularization was observed 5 days after return to room air (P17). After P21, the level of retinal neovascularization was just beginning to regress.

C. Treatment

After mice had been removed from oxygen, antisense oligonucleotides were injected into the vitreous with a Hamilton syringe and a 33 gauge needle (Hamilton Company, Reno, NV). The animals were anesthetized for the procedure with Avertin ip. The mice were given a single injection of antisense oligonucleotides (or sense or non-sense controls) at P12 achieving a final concentration of approximately 30 μM. The animals were sacrificed at P17 with tribromoethanol ip (0.1 ml/g body weight) and cervical dislocation.

D. Microscopy

The eyes were enucleated, fixed in 4% paraformaldehyde, and embedded in paraffin. Serial sections of the whole eyes were cut sagittally, through the cornea, and parallel to the optic nerve. The sections were stained with hematoxylin and periodic acid-Schiff (PAS) stain. The extent of neovascularization in the treated eyes was determined by counting endothelial cell nuclei extending past the internal limiting membrane into the vitreous. Nuclei from new vessels and vessel profiles could be distinguished from other structures in the retina and counted in cross-section with light microscopy. Additional eyes were sectioned and examined by *in situ* hybridization to a VEGF probe.

To examine the retinal vasculature using fluorescein-dextran, the mice were perfused with a 50 mg/ml solution of high molecular weight fluorescein-dextran (Sigma Chemical Company, St. Louis, MO) in 4% paraformaldehyde. The eyes were enucleated, fixed in paraformaldehyde, and flat-mounted with glycerol-gelatin. The flat-mounted retinas were viewed and photographed by fluorescence microscopy using an Olympus BX60 fluorescence microscope (Olympus America Corp., Bellingham, MA).

EXAMPLE 3
RETINOPATHY OF PREMATURITY

A. Preparation of Oligonucleotides

VEGF specific oligonucleotides are synthesized as described in EXAMPLE 2A above. Sterile and endotoxin-free oligonucleotides are diluted in Balanced Salt Solution (BSS, Alcon, Fort Worth, TX) so as to have the same pH and electrolyte concentration as the aqueous or vitreous of the eye. Emalphor EC620 (2.5%, GAF Corp.) (Bursell et al. (1993) *J. Clin. Invest.* 92:2872-2876), a petroleum product, is added to change viscosity and aid in delivery properties. Doses to achieve intravitreal concentrations ranging from 0.1 μM - 100 μM are administered depending on the severity of the retinal/ocular neovascularization. The volume delivered is between 1 μl and 1 ml depending on the volume of the eye.

B. ROP Patient Profile

The patient treated is a premature, 34 week post-conception Caucasian female weighing less than 1,000 grams at birth and is respirator-dependent. The patient has bilateral stage 3+ disease with 11 clock hours of neovascularization in each eye. There is hemorrhaging in one eye, and both eyes have reached "threshold" according to the international classification (i.e., each eye has >50% chance of going on to retinal detachment). Extraretinal fibrovascular proliferation is found in both eyes.

C. Treatment

The intubated patient is anesthetized with fluorane. The face and eyes are prepared with a betadine scrub and draped in the usual sterile fashion. The sterile drug with vehicle is injected with a 33 gauge needle on a sterile syringe at the posterior limbus (pars plana) through full thickness sclera into the vitreous. No closing suture is required unless there is

leakage. Antibiotic drops containing gentamicin or erythromycin ointment is applied to the surface of the globe in the palpebral fissure several times per day until there is complete wound closure. The frequency of injection ranges from every other day to once every 6 months or less, depending on the severity of the disease process, the degree of intraocular inflammation, the character of the vehicle (i.e., slow release characteristics), the degree of inhibition of the neovascularization and the tolerance of the eye to injections. Short and long term follow-up check-ups for possible retinal detachment from the neovascular disease as well as from the injections are necessary.

D. Monitoring of Progress

The eye upon dilation is monitored for signs of inflammation, infection, and resolution of neovascularization by both a direct and a indirect ophthalmoscope to view the retina and fundus. A slit lamp exam is used in some cases of anterior segment disease. Positive response to treatment includes fewer neovascular tufts, fewer clock hours of involvement, and less tortuosity of large blood vessels. Monitoring can be as frequent as every day in cases where premature infants are threatened with retinal detachment from proliferative ROP. The frequency of monitoring will diminish with resolution of neovascularization.

EXAMPLE 4
DIABETIC RETINOPATHY

A. Preparation of Oligonucleotides

VEGF specific oligonucleotides are synthesized as described in EXAMPLE 2A above and prepared for administration as described in EXAMPLE 3A above. Doses to achieve intravitreal concentrations ranging from 0.1 - 100 μM are administered depending on the severity of the retinal/ocular neovascularization. The volume delivered is between 1 μl and 1 ml depending on the volume of the eye and whether vitreous has

been previously removed as during a vitrectomy for diabetic eye disease.

B. Diabetic Patient Profile

The patient to be treated is a 30 year old African American male suffering for 25 years from juvenile-onset diabetes. The patient has bilateral proliferative retinopathy with sub-retinal hemorrhaging, cotton wool spots, and exudates. Upon fluorescein angiography, there are well defined areas of neovascularization bilaterally with areas of capillary drop-out.

C. Treatment

The patient is treated weekly with intraocular injections of oligonucleotides resuspended in the appropriate vehicle (BSS, Emanfour) at concentrations within the range of 0.1 - 100 μM. The treatment may be supplemented with systemic delivery of oligonucleotide (i.e., intravenous, subcutaneous, or intramuscular) from 2 to 5 times per day to once a month, depending on the disease process and the biological half life of the oligonucleotides.

D. Monitoring of Progress

The patient's eyes are monitored as described above in EXAMPLE 2D. The eyes upon dilation are examined for regression of neovascularization with both a direct and an indirect ophthalmoscope to view the retina and fundus. A slit lamp exam is used in the case of anterior segment disease. Repeat injections are given as needed, based on the degree of inhibition of the neovascularization and the tolerance of the eye to injections. Short and long term follow-up check-ups are given to check for possible retinal detachment from the neovascular disease as well as from the injections.

EXAMPLE 5
AGE-RELATED MACULAR DEGENERATION

A. Preparation of Oligonucleotides

VEGF specific oligonucleotides are synthesized as described in EXAMPLE 2A above and prepared as described in EXAMPLE 3A above. Doses to achieve intravitreal concentrations ranging from 1 μl and 1 ml are administered depending on the severity of the retinal/ocular neovascularization. The volume delivered is between 1 μl and 1 ml depending on the volume of the eye and whether vitreous has been previously removed.

B. ARMD Patient Profile

The patient is a 50 year old Caucasian male suffering from the exudative form of age related macular degeneration. This patient has choroidal neovascularization which is apparent from fluorescein angiography. The disease is bilateral and the patient has a reduction in vision in each eye from 20/60 to 20/100.

C. Treatment

The patient is treated weekly with intraocular injections of oligonucleotide resuspended in the appropriate vehicle (BSS, Emanfour) at concentrations within the range of 0.1 to 100 μM. This treatment may be supplemented with systemic delivery of oligonucleotide (i.e., intravenous, subcutaneous, or intramuscular) from 2 to 5 times per day to once a month.

D. Monitoring of Progress

The eyes upon dilation are examined for regression of neovascularization with both a direct and an indirect ophthalmoscope to view the retina and fundus. Fluorescein angiography is used to check for the resolution of

neovascularization. A slit lamp exam is used in the case of anterior segment disease. Repeat injections are given as needed, based on the degree of inhibition of the neovascularization and the tolerance of the eye to injections. Short and long term follow-up check-ups are given to check for possible retinal detachment from the neovascular disease as well as from the injections.

EXAMPLE 6
HUMAN CELL CULTURE

U373 human neuroblastoma cells were cultured in Dulbecco's modified Earls (DME) medium containing glucose (4500 mg/ml) and glutamate (2 mM) (Mediatech, Washington, DC) supplemented with penicillin/streptomycin (100 IU/MI/100 mcg/ml, Mediatech, Washington, DC). The cells were cultured at 37°C under 10% CO_2. The cells were plated in 96 well tissue culture dishes (Costar Corp., Cambridge, MA) and maintained as above. The cells were placed under anoxic conditions for 18-20 hours using an anaerobic chamber (BBL Gas Pak, Cockeysville, MD) or in the presence of 250 μM $CoCl_2$.

EXAMPLE 7
NORTHERN BLOTTING

In order to determine the level at which inhibition of VEGF expression occurs in cells in the presence of an oligonucleotide of the invention, Northern blotting was carried out. Human U373 cells cultured as described in EXAMPLE 6 above were plated in 100 mm tissue culture dishes and treated for 12 hours in the presence of 5 μg/ml lipofectin (Gibco-BRL, Gaithersburg, MD) as a lipid carrier with oligonucleotide H-3 (SEQ ID NO:6) (antisense oligonucleotide) and H-16 (SEQ ID NO:29) (sense oligonucleotide) at 0.05 μM, 0.5 μM, and 2.0 μM, respectively. The cells were refed after 12 to 15 hours with fresh media + oligonucleotide (minus lipofectin) and allowed to recover for 5 to 7 hours. The cells were placed in hypoxia for 18 to 20 hours total RNA was

isolated using the single-step acid guanidinium thiocyanate-phenol-chloroform extraction method described by Chomczynski and Sacchi (*Anal. Biochem.* (1987) **162**:156-159). Northern blotting was performed according to the methods of Sambrook et al. (*Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY) (1989) Vol. 1, pp. 7.38) or Arcellana-Panlilio et al. (*Meth. Enz.* (1993) **225**:303-328). All RNA signals were quantified on a Phosphorimager (BioRad, Hercules, CA) and normalized using the 36B4 cDNA probe (Laborda (1991) *Nucleic Acids Res.* **19**:3998). RNA expression was reduced in the presence of VEGF-specific oligonucleotides of the invention, and is not significantly affected by the presence of control sense oligonucleotide.

EXAMPLE 8

ELISA VEGF PROTEIN STUDY

U373 neuroblastoma cells as described in EXAMPLE 6 above were plated in a 96 well tissue culture dish and treated overnight with varying concentrations of antisense oligonucleotides against human VEGF in the presence of 5 μg/ml lipofectin. The cells were refed after 12 to 15 hours with fresh media + oligonucleotide (no lipofectin) and allowed to recover for 5 to 7 hours. The dishes were placed under hypoxic conditions for 18 to 20 hours using an anaerobic chamber (Gas Pac, Cockeysville, MD). The media was analyzed using the antigen capture ELISA assay described above (approximately 36 hours post treatment). The human VEGF oligonucleotides used were H-3 (SEQ ID NO:6) (antisense, coding), H-16 (SEQ ID NO:29) (start site/coding, sense control), and a random control (R).

The culture medium from the cells described in EXAMPLE 5 was analyzed for VEGF protein as follows. 96-well plates (Maxizorb ELISA Nunc A/S, Camstrup, Denmark) were treated overnight at 4°C with 100 μl/well of the capture antibody, a monoclonal antibody against human VEGF (R&D Systems, Minneapolis, MN, 2.5 μg/ml in 1X PBS). The wells were washed three times with 1X PBS/0.05%

Tween-20 (United States Biochemical, Cleveland, OH) using a plate washer (Dynatech, Gurnsey Channel Islands). Non-specific binding sites in the wells were blocked by adding 2% normal human serum (100 μl) and incubating the plate at 37°C for 2 hours. This blocking solution was removed and 200 μl conditioned medium containing human VEGF added to each well and incubated at 37°C for 2 to 3 hours. The plates were washed as described above. 100 μl of the primary antibody (618/619, 2 μg/ml in normal human serum) was added to each well and incubated at 37°C for 1 to 2 hours. The secondary antibody was an affinity purified rabbit anti- human VEGF polyclonal). The plates were washed as described above. 100 μl of the detection antibody, a horse radish peroxidase-labelled goat anti-mouse IgG monoclonal antibody (1:10,000, Vector Laboratories, Burlinggame, CA), was added to each well and incubated at 37°C for 1 hour. The plates were washed as described above. The wells were developed using the TMB microwell peroxidase developing system (Kirkegaard and Perry, Gaithersburg, MD), and quantified at 450 nm using a Ceres 900 plate reader (Bio-Tek Instruments, Inc., Winooski, Vermont). The linear range of this assay is between 2 ng and 0.01 ng human VEGF. Representative results are shown in FIG. 3.

EXAMPLE 9
BIOACTIVITY ASSAYS

Bioactivity can be determined by the Miles vessel permeability assay (Miles and Miles (1952) *J. Physiol. (Lond.)* **118**:228). Briefly, Hartley guinea pigs (800 g) are shaved and depilated and injected intravenously with 1.0 ml of normal saline containing 0.5 g of Evans Blue dye per 100 ml. Subcutaneous injections (250 μl) of serum-free medium containing unknown quantities of VEGF are performed. Positive (purified VEGF) and negative controls (normal saline) are also included in the experiment. Twenty minutes post-injection, the animals are sacrificed and the test and control sites are cut out and quantitated for extravasation of Evans Blue dye. The limit of detection for this assay is 500 pM.

Endothelial cell mitogenicity can also manifest bioactivity. In this method, human umbilical vein endothelial (HUVEC) are grown and maintained using the Biocoat endothelial cell growth environment (Collaborative Biomedical Products, Bedford, MA). 1 x 10^4 cells are then plated in duplicate on 35 mM tissue culture dishes in 1.4 ml E-STIM medium (Collaborative Biomedical Products, Bedford, MA) plus 5% heat-inactivated fetal bovine serum. Following cell attachment (about 4 hours), two dishes of cells are trypsinized, counted, and used for a starting cell number. Test samples containing unknown amounts of VEGF are then added in duplicate to the remaining dishes at day 0 and at day 2. Controls consisting of purified VEGF (positive) and PBS (negative) are also used. On day 4, the dishes of cells are trypsinized, counted and compared to the starting cell number. The limit of detection for this assay is 10 pM.

The intracellular calcium release assay is also used to monitor bioactivity (see, e.g., Brock and Capasso (1988) *J. Cell. Physiol.* 136:54). Human umbilical vein endothelial cells (HUVEC) are maintained in EGM-UV medium. Cells are removed from the plate by means of EDTA and collagenase. The calcium-sensitive dye, Fura-2 (Molecular Probes, Eugene, OR), is used to monitor changes in the concentration of intracellular calcium. In brief, medium containing an unknown concentration of VEGF is added to an aliquot of suspended HUVEC, pre-loaded with Fura-2. Changes in fluorescence representing changes in intracellular calcium release are measured using a Hitachi 2000 F fluorometer. Positive (histamine, thrombin) and negative (EGTA) controls are also analyzed. This method is extremely sensitive and has a limit of detection of 0.2 pM.

EXAMPLE 10
ELISA VEGF PROTEIN STUDY

U373 glioblastoma cells were plated in a 96 well tissue culture dish and treated overnight with varying concentrations of antisense oligonucleotides against human VEGF in the presence

of 5 μg/ml lipofectin. The cells were refed after 7 to 12 hours with fresh media and allowed to recover for 5 to 7 hours. The dishes were placed under hypoxic conditions for 18 to 20 hours using an anaerobic chamber (Gas Pac, Cockeysville, MD) or in the presence of 250 μM $CoCl_2$. Cells maintained under normoxic conditions served as uninduced controls. The media was analyzed using the antigen capture ELISA assay described below (approximately 24 hours post treatment).

The culture medium from the cells described in EXAMPLE 2 was analyzed for VEGF protein as follows. 96-well plates (Maxizorb ELISA Nunc A/S, Camstrup, Denmark) were treated overnight at 4°C with 100 μl/well of the capture antibody, a monoclonal antibody against human VEGF (R&D Systems, Minneapolis, MN, 2.5 μg/ml in 1X PBS). The wells were washed three times with 1x PBS/0.05% Tween-20 (United States Biochemical, Cleveland, OH) using a plate washer (Dynatech, Gurnsey Channel Islands). Non-specific binding sites in the wells were blocked by adding 2% normal human serum (200 μl) and incubating the plate at 37°C for 2 hours. This blocking solution was removed and 200 μl conditioned medium containing human VEGF added to each well and incubated at 37°C for 2 to 3 hours or overnight at 4°C. The plates were washed as described above. 100 μl of the primary antibody (618/619, 2 μg/ml in normal human serum) was added to each well and incubated at 37°C for 1 to 2 hours. The primary antibody was an affinity purified rabbit anti-human VEGF polyclonal). The plates were washed as described above. 100 μl of the detection antibody, a horse radish peroxidase-labelled goat anti-rabbit IgG monoclonal antibody (1:10,000, Vector Laboratories, Burlinggame, CA), was added to each well and incubated at 37°C for 1 hour. The plates were washed as described above. The wells were developed using the TMB microwell peroxidase developing system (Kirkegaard and Perry, Gaithersburg, MD), and quantified at 450 nm using a Ceres 900 plate reader (Bio-Tek Instruments, Inc., Winooski, Vermont). The linear range of this assay is between 2 ng and 0.01 ng human VEGF. Representative results are shown in FIGS. 2-6.

EXAMPLE 11
IN VIVO STUDIES

A. Matrigel Studies

U373 glioblastoma cells were treated with 0.5 μM antisense phosphorothioate oligodeoxynucleotide (H-3, SEQ ID NO:54) or control (H3-sense phosphorothioate oligonucleotide; SEQ ID NO:74) for 7 hours in the presence of 5 μg/μl Lipofectin (Gibco-BRL, Gaithersburg, MD). 1 x 10^6 oligonucleotide-treated cells were mixed with 250 μl Matrigel™ (Collaborative Research, Waltham, MA; 10-12 mg/ml) and injected subcutaneously into 6-8 week old athymic mice (about 20 g) (Charles River Laboratories, Wilmington, MA) on both the left and right sides. These cells respond to hypoxia and express increased levels of VEGF. The mice were maintained *ad libitum* and sacrificed 8 days post injection. The skin was dissected to expose the Matrigel pellet. Gross photography of the surrounding blood vessels was performed with a Zeiss Macroscope. The Matrigel plugs were removed and fixed in formalin for paraffin embedding and histological analysis. Tissue sections were stained with hematoxylin and eosin for quantitation of blood vessel growth into the Matrigel plug.

The injection of Matrigel alone resulted in a clear plug with no apparent angiogenesis. Matrigel plugs combined with U373 glioblastoma cells contained visible hemhorraging. In addition, the capillary bed surrounding the plug was more dense and the blood vessels were more tortuous. Athymic mice injected with Matrigel plugs combined with antisense oligonucleotide-treated cells generated less angiogenesis than the mice injected with Matrigel plugs and either untreated cells or cells pretreated with the control oligonucleotide. Matrigel plugs containing antisense treated cells also had less visible hemhorraging. The results suggest that antisense oligonucleotide treatment inhibit VEGF-induced angiogenesis.

B. Tumor Studies

6 week old athymic mice (about 20 g) are purchased from Charles River Laboratories. Human melanoma M21 cells or human glioblastoma U373 cells which have been passaged through athymic mice in the presence of Matrigel are injected subcutaneously (2-20 x 10^6) into the flank of athymic mice. Palpable tumors are generated in 1-2 weeks. Subcutaneous antisense or sense control oligonucleotide injections begin one day following the injection of the tumor cells. The concentration of oligonucleotide is determined and ranges between 5 and 50 mg/kg. Animals are then injected over a period of three weeks. They are then sacrificed and the tumors removed. Tumors are analyzed initially for weight and volume. In addition, analysis includes sectioning and staining for VEGF/VPF protein using an anti- human VEGF/VPF monoclonal antibody (R&D Systems, Minneapolis, MN) or VEGF/VPF RNA using *in situ* hybridization techniques. Mice injected with antisense oligonucleotides of the invention are expected to have smaller tumors than those injected with vehicle or the control.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hybridon, Inc.

(ii) TITLE OF INVENTION: Human VEGF-Specific Oligonucleotides

(iii) NUMBER OF SEQUENCES: 74

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 200 State Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kerner, Ann-Louise
(B) REGISTRATION NUMBER: 33,523
(C) REFERENCE/DOCKET NUMBER: HYZ-031CPPCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-330-1300
(B) TELEFAX: 617-330-1311

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCCGGGCCG CCAGCACACT 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGCCGGGCCG CCAGCACACU 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCCGCCAGC ACACT 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTCGCGCCG GGCCGCCAGC ACACT 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAAGACAGCA GAAAGTTCAT 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACCCAAGAC AGCAGAAAG 19

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACCCAAGAC AGCAG 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCAATGCACC CAAGACAGCA GAAAG 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGCACACAG AACAAGACG 19

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AAGTTCATGG TTTCGGAGGC 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTTCATGGTT TCGGAGGCCC 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGCAGCCTG GGACCACTTG 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCCTCGGCT TGTCACATCT 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTCCTCCTG CCCGGCTCAC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CUUCCUCCUG CCCGGCUCAC 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTTCCTCCTG CCCGG 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGCTCCTTCC TCCTGCCCGG CTCAC 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCTCCTCTT CCTTCATTTC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTCTCCTCTT CCTTC 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCAGAGTCTC CTCTTCCTTC ATTTC 25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGGACCCAAA GTGCTCTGCG 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCAAAGTGCT CTGCG 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCCTCCGGAC CCAAAGTGCT CTGCG 25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGCACGACC GCTTACCTTG 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGACCACTG AGGACAGAAA 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CACCACTGCA TGAGAGGCGA 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCCCAAAGAT GCCCACCTGC 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGCATAATCT GGAAAGGAAG 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACTTTCTGCT GTCTTGGGTG 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CATGGTTTCG GAGGCCCGAC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CAUGGTTUCG GAGGCCCGAC 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TTCATGGTTT CGGAGGCCCG 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GACCGCTTAC CTTGGCATGG 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCTGGGACCA CTGAGGACAG 20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGACTCACC TTCGTGATGA 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GAACTTCACC ACTGCATGAG 20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TCCCAAAGAT GCCCACCTGC 20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCATAATCTG GAAAGGAAGG 20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ACATCCTCAC CTGCATTCAC 20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ACATCCUCAC CTGCAUUCAC 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TTTCTTTGGT CTGCAATGGG 20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGCCACTTAC TTTTCTTGTC 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CACAGGGACT GGAAAATAAA 20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGGAACCAAC CTGCAAGTAC 20

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTCACATCTG AGGGAAATGG 20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CAGCCTGGCT CACCGCCTTG G 21

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TCGCGCTCCC TCTCTCCGGC 20

(2) INFORMATION FOR SEQ ID NO:48

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CATGGTTTCG GAGGGCGTC 19

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TCCGAAACCA TGAACTTTCT G 21

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGTTTCGGAG GCCCGACCG 19

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CAAGAGAGCA GAAAGTTCAT 20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CACCCAAGAG AGCAGAAACT 20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TCGTGGGTGC AGCCTGGGAC 20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACCCAAGAC AGCAGAAAG 19

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCACCCAAGA CAGCAGAAAG 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGCACCCAAG ACAGCAGAAA G 21

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATGCACCCAA GACAGCAGAA AG 22

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

AATGCACCCA AGACAGCAGA AAG 23

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CAATGCACCC AAGACAGCAG AAAG 24

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCAATGCACC CAAGACAGCA GAAAG 25

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCCAATGCAC CCAAGACAGC AGAAAG 26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CTCCAATGCA CCCAAGACAG CAGAAAG 27

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTCCAATGC ACCCAAGACA GCAGAAAG 28

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGCTCCAATG CACCCAAGAC AGCAGAAAG 29

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CACCCAAGAC AGCAGAAA 18

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CACCCAAGAC AGCAGAA 17

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CACCCAAGAC AGCAGA 16

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CACCCAAGAC AGCAGAAAGT T 21

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CACCCAAGAC AGCAGAAAGT TCAT 24

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

TCGCGCTCCC TCTCTCCGGC 20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CATGGTTTCG GAGGGCGTC 19

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CAGCCTGGCT CACCGCCTTG G 21

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

TCCGAAACCA TGAACTTTCT G 21

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTTCTGCTG TCTTGGGTG 19

What is claimed is:

1. A synthetic oligonucleotide complementary to a nucleic acid specific for human vascular endothelial growth factor.

2. The oligonucleotide of claim 1 having SEQ ID NO:1.

3. The oligonucleotide of claim 1 having SEQ ID NO:2.

4. The oligonucleotide of claim 1 having SEQ ID NO:3.

5. The oligonucleotide of claim 1 having SEQ ID NO:4.

6. The oligonucleotide of claim 1 having SEQ ID NO:5.

7. The oligonucleotide of claim 1 having SEQ ID NO:6.

8. The oligonucleotide of claim 1 having SEQ ID NO:7.

9. The oligonucleotide of claim 1 having SEQ ID NO:8.

10. The oligonucleotide of claim 1 having SEQ ID NO:9.

11. The oligonucleotide of claim 1 having SEQ ID NO:10.

12. The oligonucleotide of claim 1 having SEQ ID NO:11.

13. The oligonucleotide of claim 1 having SEQ ID NO:12.

14. The oligonucleotide of claim 1 having SEQ ID NO:13.

15. The oligonucleotide of claim 1 having SEQ ID NO:14.

16. The oligonucleotide of claim 1 having SEQ ID NO:15.

17. The oligonucleotide of claim 1 having SEQ ID NO:16.

18. The oligonucleotide of claim 1 having SEQ ID NO:17.

19. The oligonucleotide of claim 1 having SEQ ID NO:18.

20. The oligonucleotide of claim 1 having SEQ ID NO:19.

21. The oligonucleotide of claim 1 having SEQ ID NO:20.

22. The oligonucleotide of claim 1 having SEQ ID NO:21.

23. The oligonucleotide of claim 1 having SEQ ID NO:22.

24. The oligonucleotide of claim 1 having SEQ ID NO:23.

25. The oligonucleotide of claim 1 having SEQ ID NO:24.

26. The oligonucleotide of claim 1 having SEQ ID NO:25.

27. The oligonucleotide of claim 1 having SEQ ID NO:26.

28. A synthetic oligonucleotide complementary to a nucleic acid specific for human vascular endothelial growth factor selected from the group consisting of an oligonucleotide having SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:67, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, and SEQ ID NO:69.

29. The oligonucleotide of claim 28 having SEQ ID NO:55.

30. The oligonucleotide of claim 28 having SEQ ID NO:56.

31. The oligonucleotide of claim 28 having SEQ ID NO:57.

32. The oligonucleotide of claim 28 having SEQ ID NO:58.

33. The oligonucleotide of claim 28 having SEQ ID NO:59.

34. The oligonucleotide of claim 28 having SEQ ID NO:60.

35. The oligonucleotide of claim 28 having SEQ ID NO:61.

36. The oligonucleotide of claim 28 having SEQ ID NO:62.

37. The oligonucleotide of claim 28 having SEQ ID NO:63.

38. The oligonucleotide of claim 28 having SEQ ID NO:64.

39. The oligonucleotide of claim 28 having SEQ ID NO:65.

40. The oligonucleotide of claim 28 having SEQ ID NO:66.

41. The oligonucleotide of claim 28 having SEQ ID NO:67.

42. The oligonucleotide of claim 28 having SEQ ID NO:68.

43. The oligonucleotide of claim 28 having SEQ ID NO:69.

44. The oligonucleotide of claim 28 having a modification selected from the group consisting of an alkylphosphonate, phosphorothioate, phosphorodithioate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, and carboxymethyl ester internucleotide linkage, and a combination thereof.

45. The oligonucleotide of claim 44 having at least one phosphorothioate internucleotide linkage.

46. The oligonucleotide of claim 45 having phosphorothioate internucleotide linkages.

47. The oligonucleotide of claim 28 consisting essentially of 2'-O-alkylated ribonucleotides.

48. The oligonucleotide of claim 28 comprising four or five 5' 2'-O-alkylated ribonucleotides.

49. The oligonucleotide of claim 28 comprising four or five 3' 2'-O-alkylated ribonucleotides.

50. The oligonucleotide of claim 48 comprising four or five 3' 2'-O-alkylated ribonucleotides.

51. The oligonucleotide of claim 45 comprising four or five 5' 2'-O-alkylated ribonucleotides.

52. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 2.

53. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 3.

54. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 4.

55. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 5.

56. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 6.

57. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 7.

58. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 8.

59. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 9.

60. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 10.

61. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 11.

62. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 12.

63. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 13.

64. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 14.

65. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 15.

66. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 16.

67. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 17.

68. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 18.

69. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 19.

70. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 20.

71. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 21.

72. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 22.

73. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 23.

74. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 24.

75. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 25.

76. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 26.

77. A method for inhibiting VEGF expression comprising the step of providing an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 27.

78. A method of inhibiting VEGF expression comprising the step of contacting nucleic acid specific for VEGF with an oligonucleotide of claim 28.

79. A method of inhibiting VEGF expression comprising the step of contacting nucleic acid specific for VEGF with an oligonucleotide of claim 44.

80. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 2.

81. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 3.

82. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 4.

83. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 5.

84. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 6.

85. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 7.

86. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 8.

87. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 9.

88. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 10.

89. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 11.

90. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 12.

91. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 13.

92. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 14.

93. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 15.

94. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 16.

95. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 17.

96. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 18.

97. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 19.

98. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 20.

99. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 21.

100. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 22.

101. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 23.

102. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 24.

103. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 25.

104. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 26.

105. A pharmaceutical composition comprising an effective VEGF expression-inhibiting amount of the synthetic oligonucleotide of claim 27.

106. A pharmaceutical composition comprising at least one synthetic oligonucleotide of claim 28 in a physiologically acceptable carrier.

107. A pharmaceutical composition comprising at least one synthetic oligonucleotide of claim 44 in a physiologically acceptable carrier.

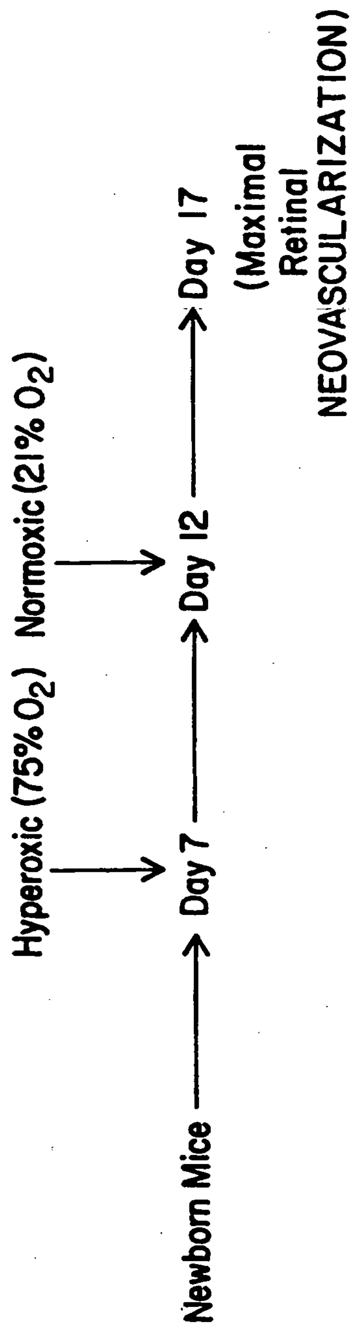

Hyperoxic (75% O_2)
Normoxic (21% O_2)
Newborn Mice
Day 7
Day 12
Day 17
(Maximal
Retinal
NEOVASCULARIZATION)

FIG. 1

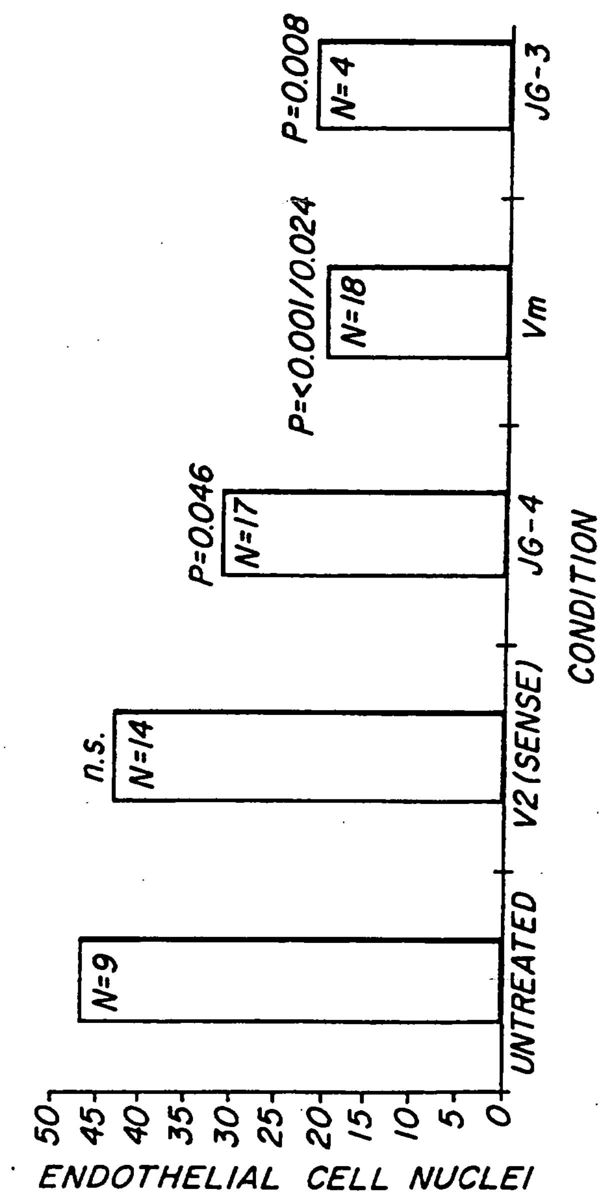

ENDOTHELIAL CELL NUCLEI
50
45
40
35
30
25
20
15
10
5
0
N=9
n.s.
N=14
P=0.046
N=17
P=<0.001/0.024
N=18
P=0.008
N=4
UNTREATED
V2 (SENSE)
JG-4
Vm
JG-3
CONDITION

FIG. 2

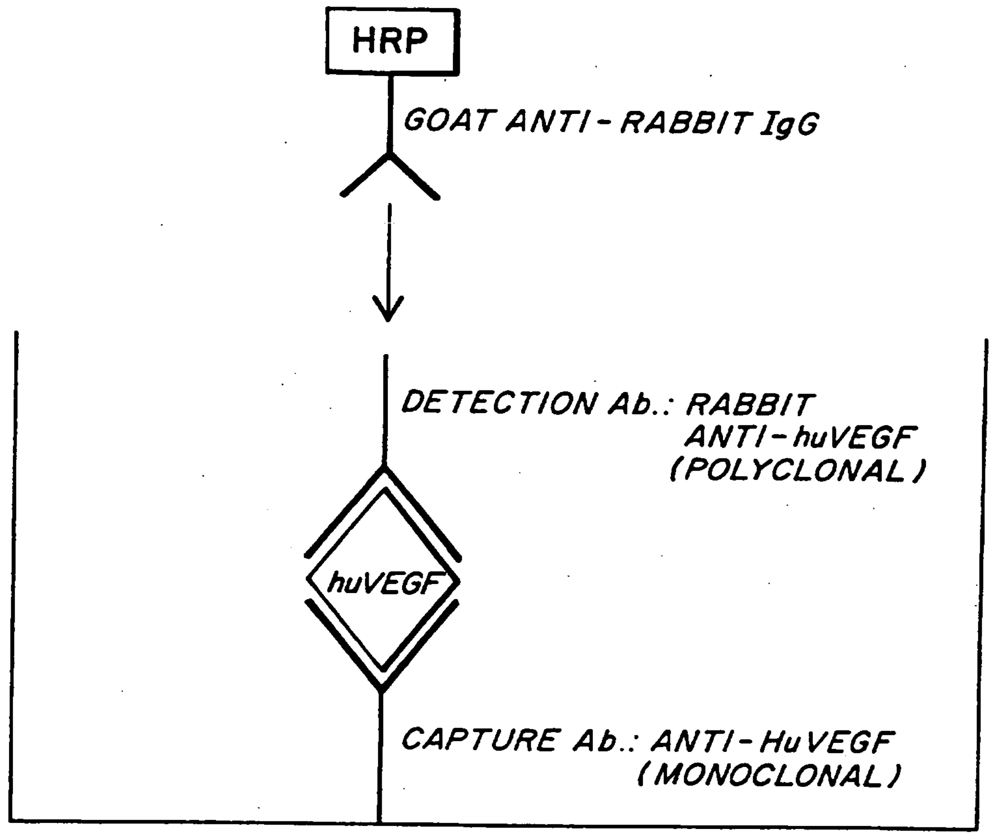

HRP
GOAT ANTI-RABBIT IgG
DETECTION Ab.: RABBIT ANTI-huVEGF (POLYCLONAL)
huVEGF
CAPTURE Ab.: ANTI-HuVEGF (MONOCLONAL)

FIG. 3

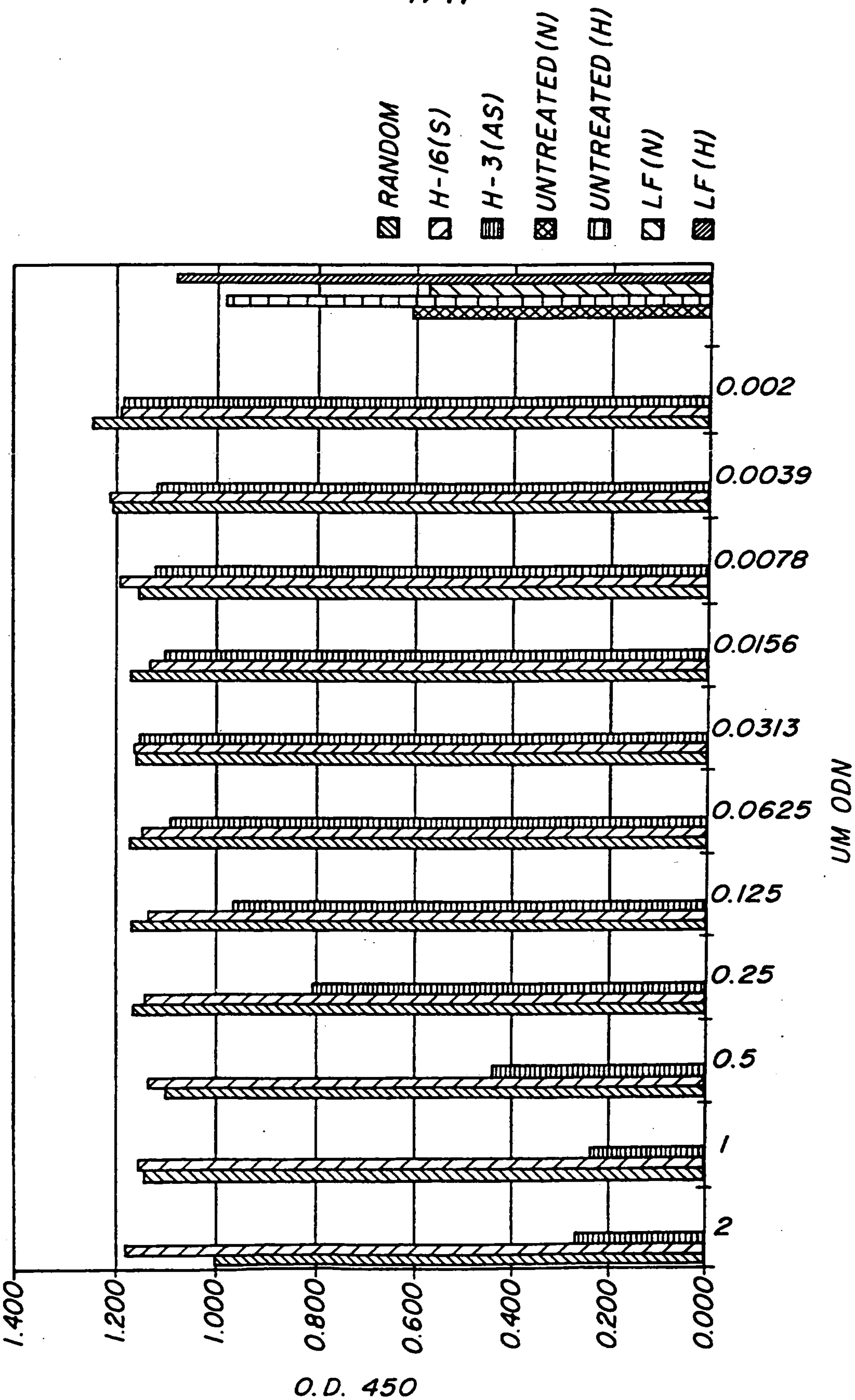

RANDOM
H-16(S)
H-3(AS)
UNTREATED(N)
UNTREATED(H)
LF(N)
LF(H)
0.002
0.0039
0.0078
0.0156
0.0313
0.0625
0.125
0.25
0.5
1
2
UM ODN
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
O.D. 450

FIG. 4

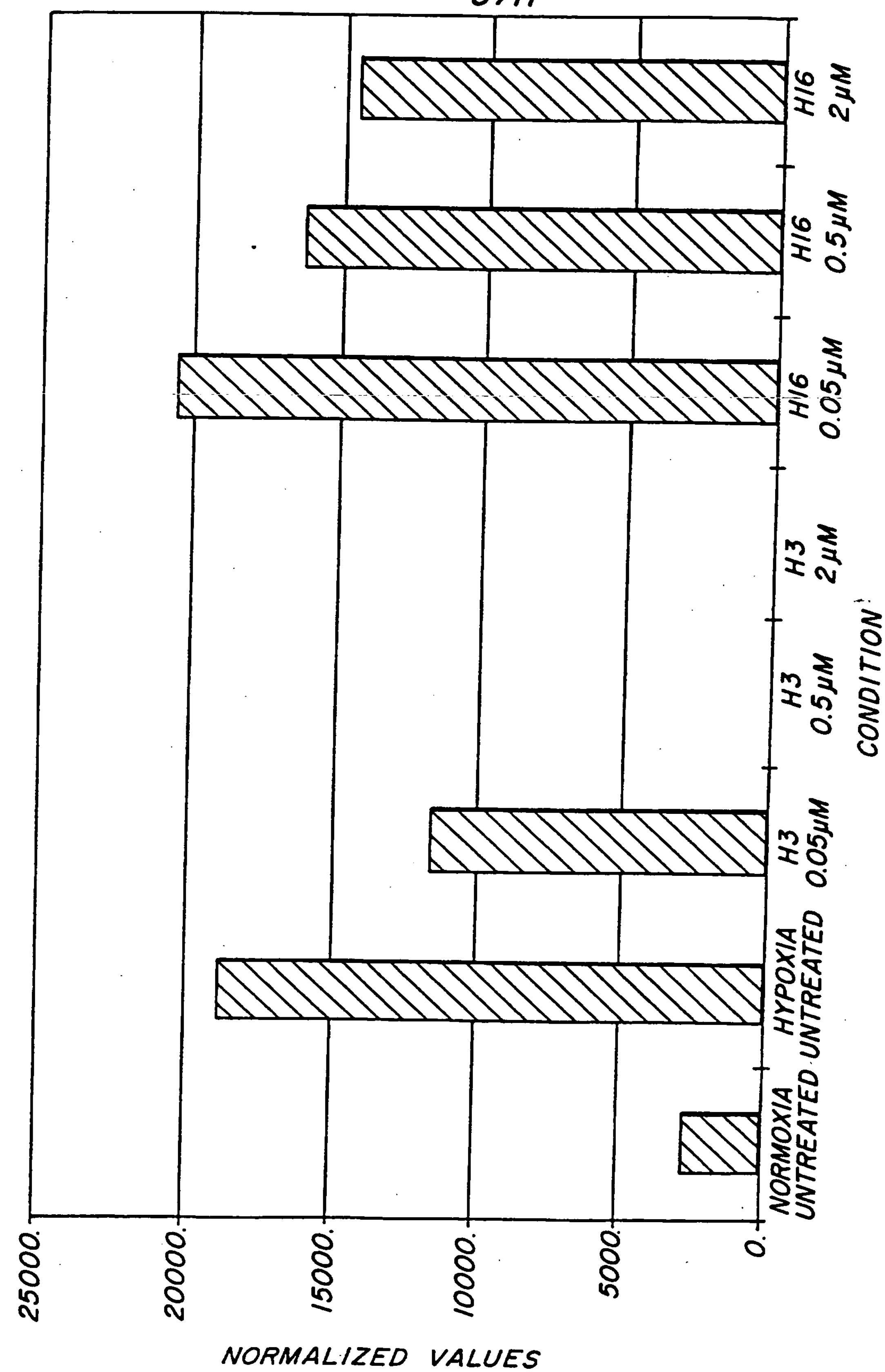

NORMALIZED VALUES
25000.
20000.
15000.
10000.
5000.
0.
CONDITION
NORMOXIA UNTREATED
HYPOXIA UNTREATED
H3 0.05μM
H3 0.5μM
H3 2μM
H16 0.05μM
H16 0.5μM
H16 2μM

FIG. 5

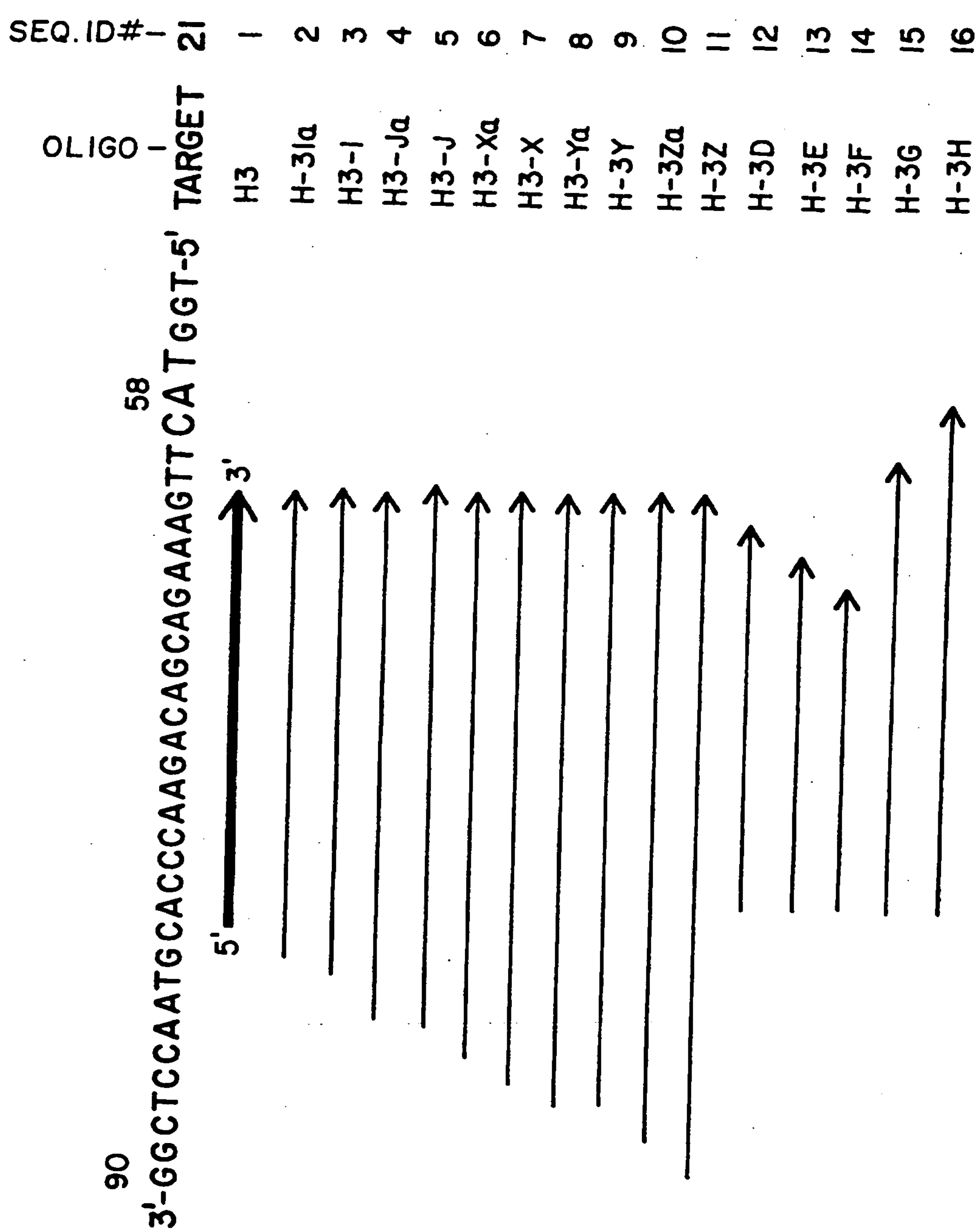

SEQ. ID#– 21 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
OLIGO – TARGET H3 H-3Ia H3-I H3-Ja H3-J H3-Xa H3-X H3-Ya H-3Y H-3Za H-3Z H-3D H-3E H-3F H-3G H-3H
90
58
3'-GGCTCCAATGCACCCAAGACAGCAGCAGAAAGTTCATGGT-5'
5'
3'

FIG. 6

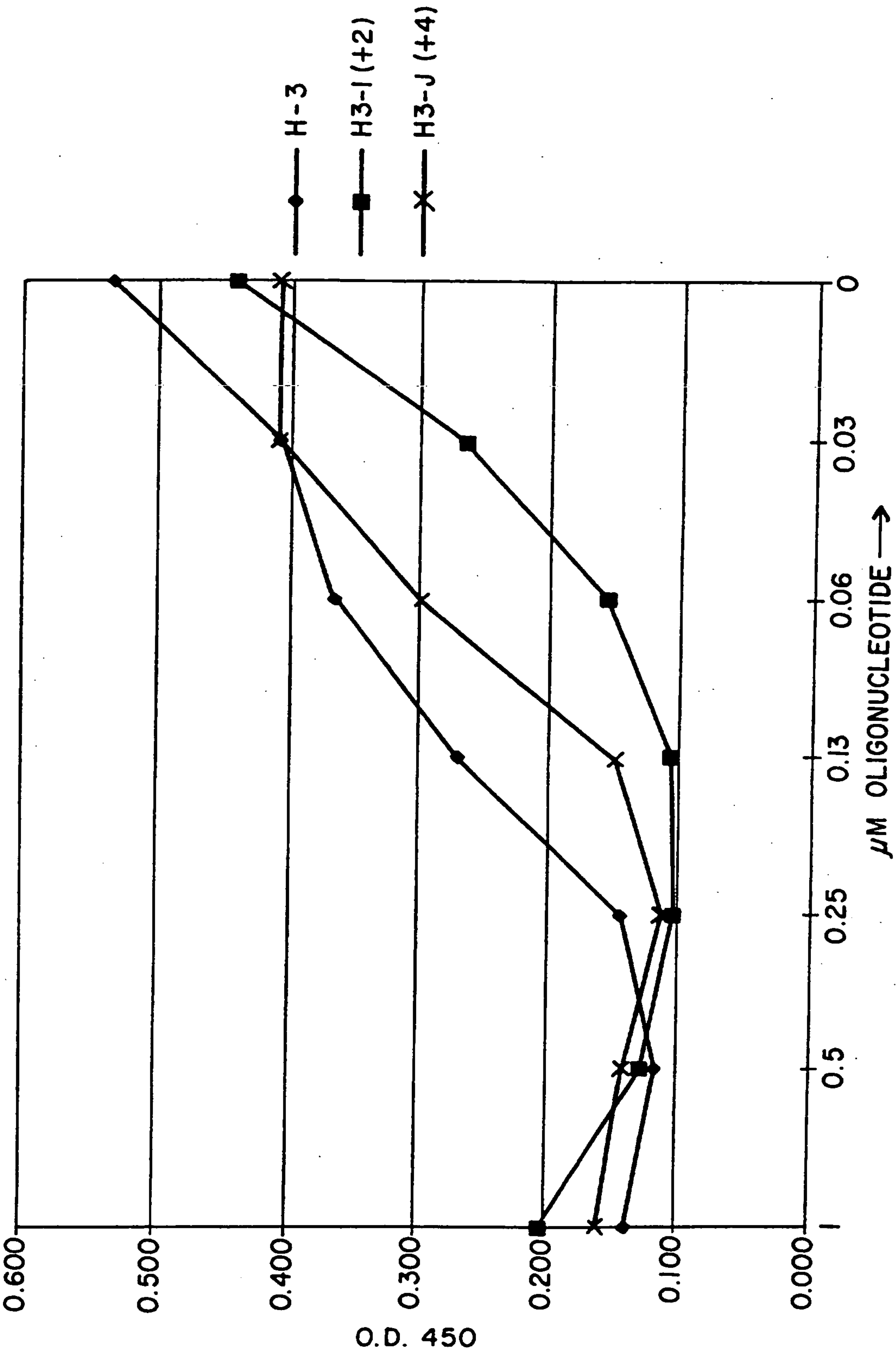
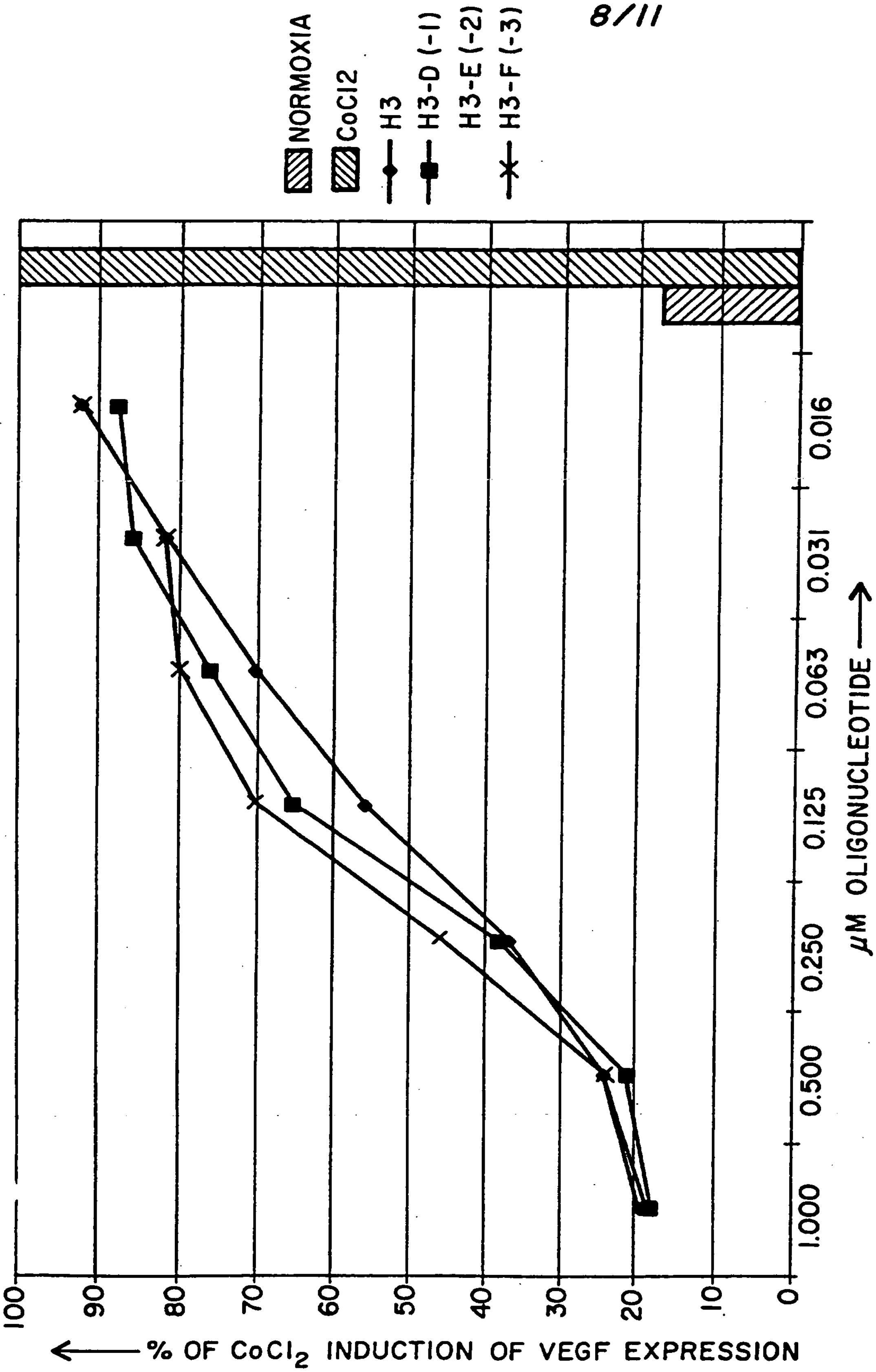

H-3
H3-I (+2)
H3-J (+4)
0.600
0.500
0.400
0.300
0.200
0.100
0.000
O.D. 450
0
0.03
0.06
0.13
0.25
0.5
1
μM OLIGONUCLEOTIDE →

FIG. 7



NORMOXIA
CoCl2
H3
H3-D (-1)
H3-E (-2)
H3-F (-3)
0.016
0.031
0.063
0.125
0.250
0.500
1.000
μM OLIGONUCLEOTIDE
100
90
80
70
60
50
40
30
20
10
0
% OF $CoCl_2$ INDUCTION OF VEGF EXPRESSION

FIG. 8

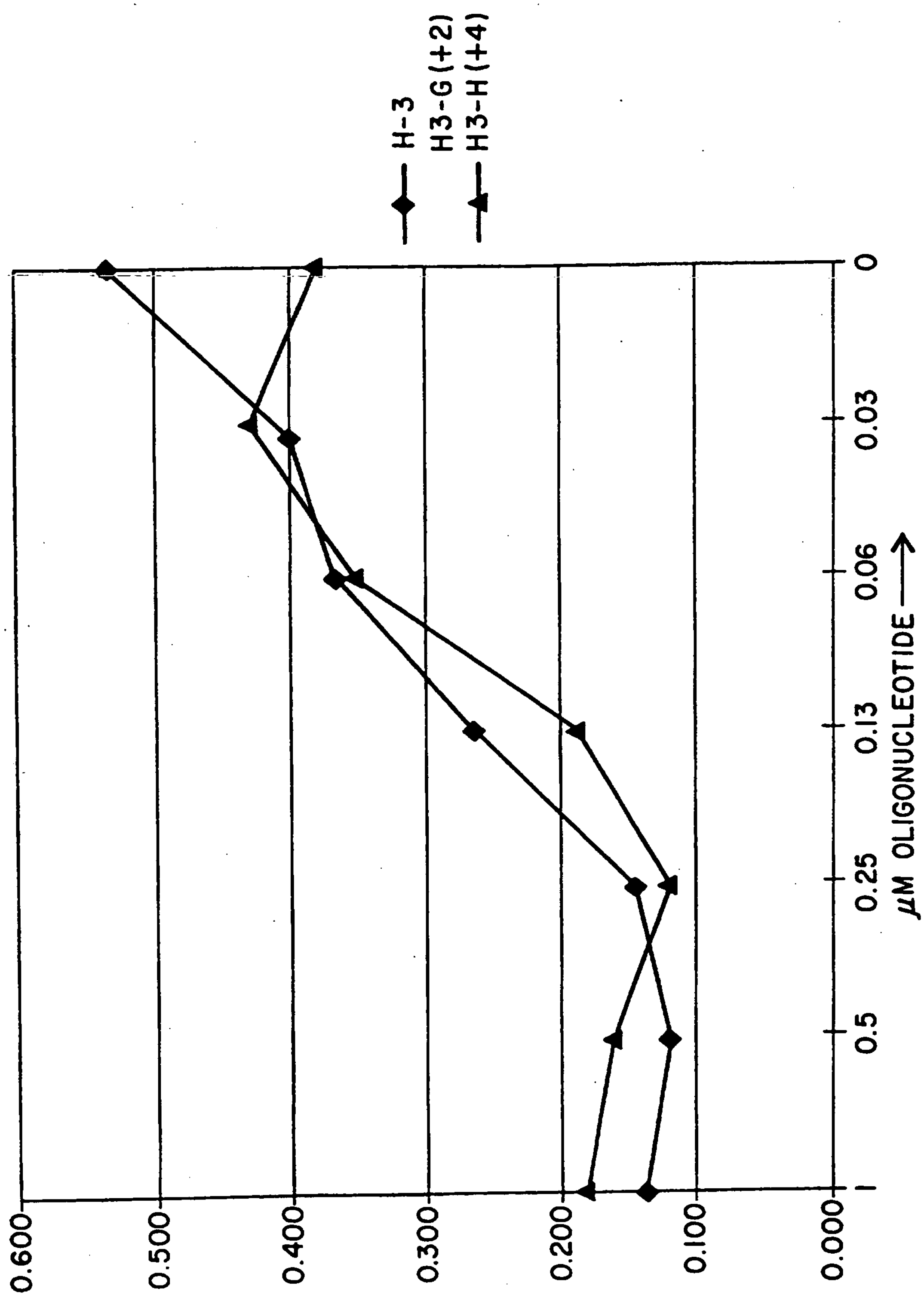

H-3
H3-G(+2)
H3-H(+4)
0.600
0.500
0.400
0.300
0.200
0.100
0.000
O.D. 450
0
0.03
0.06
0.13
0.25
0.5
1
μM OLIGONUCLEOTIDE

FIG. 9

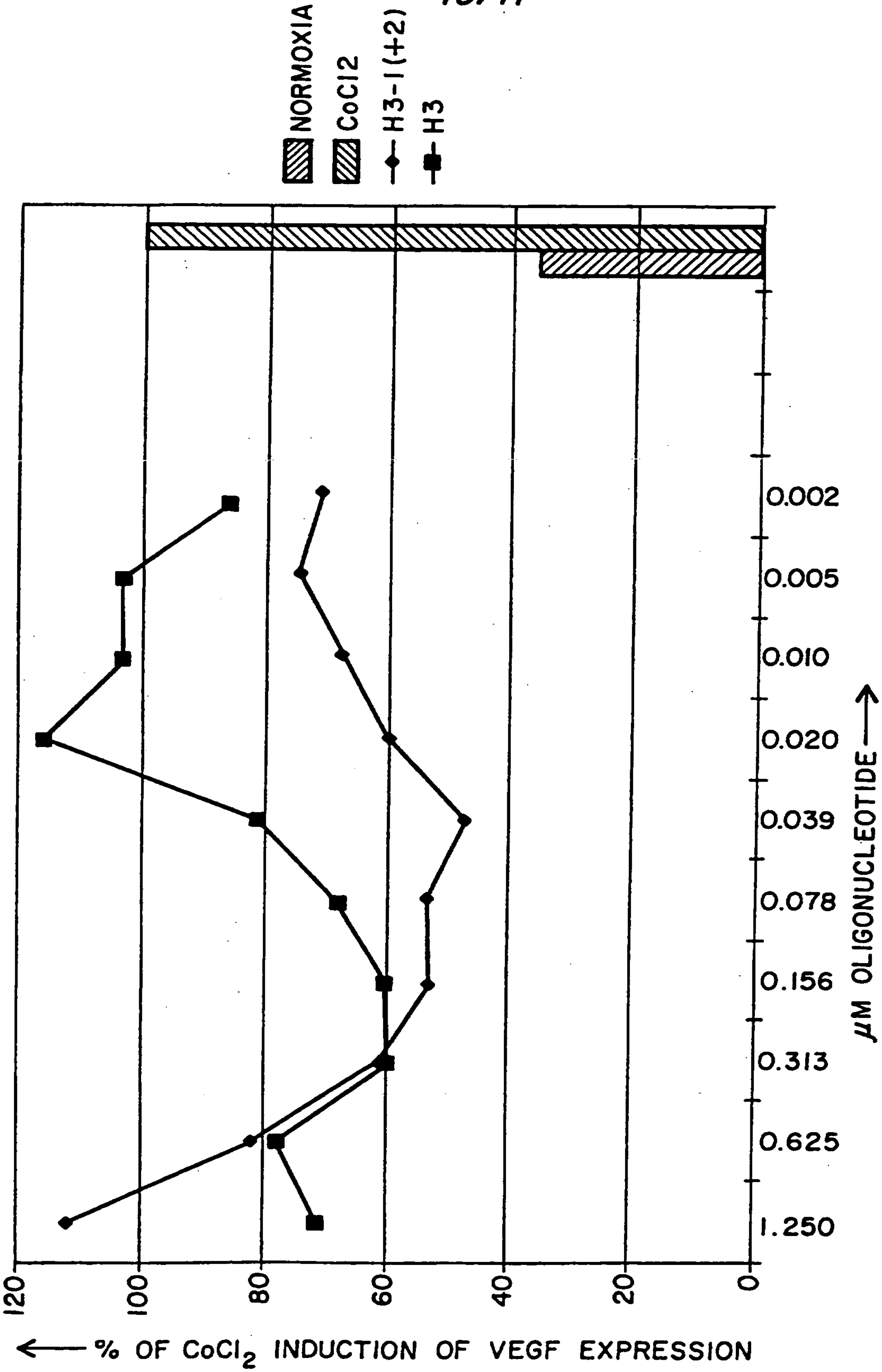

NORMOXIA
CoCl2
H3-1(+2)
H3
0.002
0.005
0.010
0.020
0.039
0.078
0.156
0.313
0.625
1.250
μM OLIGONUCLEOTIDE ⟶
120
100
80
60
40
20
0
⟵ % OF $CoCl_2$ INDUCTION OF VEGF EXPRESSION

FIG. 10

11/11

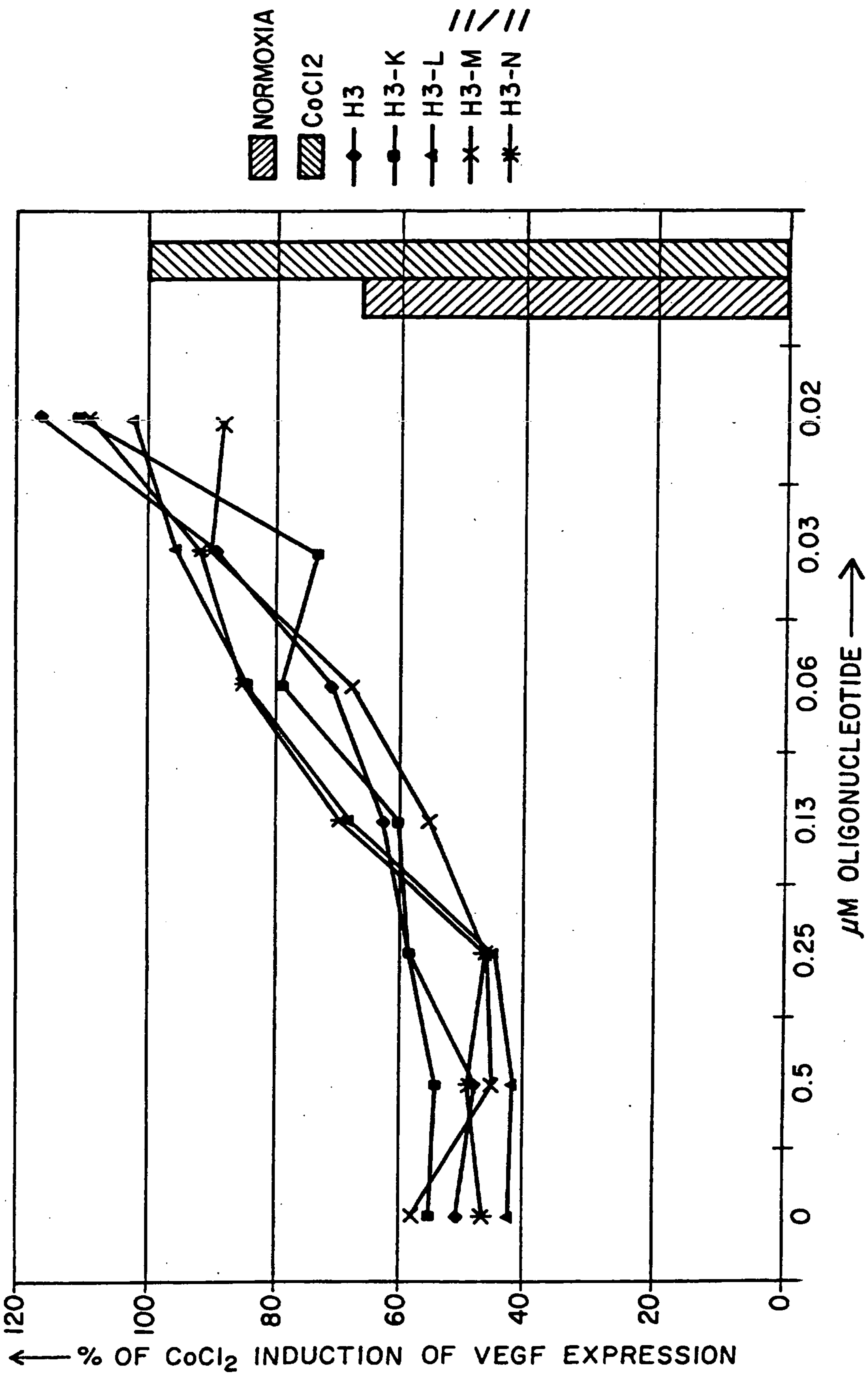

NORMOXIA
CoCl2
H3
H3-K
H3-L
H3-M
H3-N
120
100
80
60
40
20
0
← % OF $CoCl_2$ INDUCTION OF VEGF EXPRESSION
0.02
0.03
0.06
0.13
0.25
0.5
0
μM OLIGONUCLEOTIDE →

FIG. 11

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/11, A61K 31/70, C07H 21/00

A3

(11) International Publication Number: WO 96/27006

(43) International Publication Date: 6 September 1996 (06.09.96)

(21) International Application Number: PCT/US96/02840

(22) International Filing Date: 29 February 1996 (29.02.96)

(30) Priority Data:

08/398,945	2 March 1995 (02.03.95)	US
08/569,926	8 December 1995 (08.12.95)	US

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(72) Inventor: ROBINSON, Gregory, S.; 194 School Street, Acton, MA 01720 (US).

(74) Agents: KERNER, Ann-Louise et al.; Lappin & Kusmer, Two Hundred State Street, Boston, MA 02109 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

~~Before the expiration of the time~~ limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 17 October 1996 (17.10.96)

(54) Title: HUMAN VEGF-SPECIFIC ANTISENSE OLIGONUCLEOTIDES

(57) Abstract

Disclosed are oligonucleotides complementary to VEGF-specific nucleic acid useful in reducing the expression of VEGF. Also disclosed are pharmaceutical formulations containing such oligonucleotides and method useful for treating various disorders associated with neovascularization and angiogenesis.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/02840

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,95 04142 (HYBRIDON INC ;ROBINSON GREGORY S (US)) 9 February 1995	1,6,7, 28,40, 44-51, 56,57, 78,79, 84,85, 106,107
Y	see the whole document	2-9, 11-59, 61-107

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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2

Date of the actual completion of the international search	Date of mailing of the international search report
20 August 1996	30.08.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Andres, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	ANTISENSE RES.DEV. 5 (SPRING 95);87-8;OP-10 , XP002006442 UCHIDA, K. ET AL.: "Selection of antisense oligodeoxyribonucleotides that inhibit VEGF/VPF expression in a cell-free system" cited in the application	1
Y	see abstract	2-9, 11-59, 61-107
	& 1ST INTERNATIONAL ANTISENSE CONFERENCE IN JAPAN , 4 - 7 December 1994, ---	
X	AMERICAN JOURNAL OF PHYSIOLOGY, vol. 264, no. 4, April 1993, pages c995-c1002, XP002007149 MONACCI, W. ET AL.: "Expression of vascular permeability factor/vascular endothelial growth factor in normal rat tissues" see page C996, left-hand column, line 6 - line 27 ---	1
X	GROWTH FACTORS, vol. 8, 1993, pages 109-117, XP002007150 GARRIDO, C. ET AL.: "Transcriptional regulation of vascular endothelial growth factor gene expression in ovarian bovine granulosa cells" see page 111, left-hand column, line 30 - line 44 ---	1
A	JOURNAL OF BIOLOGICAL CHEMISTRY , vol. 266, 25 June 1991, MD US, pages 11947-11954, XP002007151 TISCHER, E. ET AL.: "The human gene for vascular endothelial growth factor" cited in the application ---	
A	CHEMICAL REVIEWS, vol. 90, no. 4, 1 June 1990, pages 543-584, XP000141412 UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" cited in the application ---	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 193, 15 June 1993, ORLANDO, FL US, pages 631-638, XP002007152 ADAMIS, A. ET AL.: "Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells" cited in the application	
E	WO,A,96 23065 (HYBRIDON INC ;CHILDRENS MEDICAL CENTER (US)) 1 August 1996 see the whole document	1-7,9, 11-41, 44-57, 59, 61-85, 87, 89-107

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/02840

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 52-59,69-71
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims (as far as in vivo methods are concerned) are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 7 inventions - see continuation-sheet PCT/ISA/210 -

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

- see inventions 1,

2,4-7

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US96/02840

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

YES - 1) Claims 1 (partially) and 2-5,52-55,80-83 (totally)

Oligonucleotides complementary to a nucleic acid for VEGF between nucleotides 2 and 21 and defined by SEQ ID's 1 to 4, their use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising them.

NO - 2) Claims 1 (partially) and 6-9,11-12,28-51,56-59,61-62,78-79, 84-87,89-90,106-107 (totally)

Oligonucleotides complementary to a nucleic acid for VEGF between nucleotides 43 and 90, and defined by SEQ ID's 5 to 8, 10-11 and 55-69, modified forms thereof, their use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising them.

NO - 3) Claims 1 (partially) and 10,60,88 (totally)

Oligonucleotide defined by SEQ ID 9 , its use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising it.

NO - 4) Claims 1 (partially) and 13,63,91 (totally)

Oligonucleotide complementary to a nucleic acid for VEGF between nucleotides 119 and 138, and defined by SEQ ID 12, their use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising them.

NO - 5) Claims 1 (partially) and 14-18,64-68,92-96 (totally)

Oligonucleotides complementary to a nucleic acid for VEGF between nucleotides 609 and 653, and defined by SEQ ID's 13 to 17, their use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising them.

NO - 6) Claims 1 (partially) and 19-24,69-74,97-102 (totally)

Oligonucleotides complementary to a nucleic acid for VEGF between nucleotides 827 and 779, and defined by SEQ ID's 18 to 23, their use in a method for inhibiting VEGF expression and a pharmaceutical composition comprising them.

NO - 7) Claims 1 (partially) and 25-27,75-77,103-105 (totally)

Oligonucleotides complementary to a nucleic acid for VEGF at the intron/exon boundaries, and defined by SEQ ID's 24 to 26, their use in a method for inhibiting VEGF xpression and a pharmaceutical composition comprising them.

FURTHER INFORMATION CONTINUED FR M PCT/ISA/210

1) In claim 10, the oligonucleotide defined by SEQ ID 9 is claimed as an antisense for VEGF. This oligonucleotide is not present in table 1 giving a list of VEGF-antisenses, and is described as being NOT antisense in the sequence listing (see SEQ ID 9) !
As there is no other indication in the application allowing a sure judgment on the nature and location of the oligonucleotide defined by SEQ ID 9, the ISA had no possibility to include it in one of the existing groups of inventions and had to consider claim 10 and depending claims as a distinct invention.

2) In claim 28, SEQ ID 67 is mentionhed twice whereas SEQ ID 62 is missing. Considering dependent claim 36, one of SEQ ID 67's would be read as being SEQ ID 62 if the corresponding invention was chosen for an additional search.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/02840

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9504142	09-02-95	AU-B-	7516894	28-02-95
		CA-A-	2167680	09-02-95
		EP-A-	0711343	15-05-96
		FI-A-	960374	25-03-96
		NO-A-	960303	13-03-96
WO-A-9623065	01-08-96	NONE		

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